ABI PRISM® SeqScape® Software Version 2.0

User Guide

Applied Biosystems

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SeqScape software has not undergone specific validation for human identification applications. Human identification laboratories must perform their own validation studies.

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Preface

How to Use This Guide

Purpose of This Guide

The Applied Biosystems *ABI PRISM® SeqScape® Software Version 2.0 User Guide* provides step-by-step instructions to use this software.

Audience

This guide is intended for novice and experienced analysts and scientists who are doing resequencing.

Assumptions

This manual uses conventions and terminology that assume a working knowledge of the Windows® operating system, the Internet, and Web-based browsers.

What You Should Know Before Getting Started

To make the best use of SeqScape® Software Version 2.0 and documentation, be sure you are familiar with:

- Microsoft® Windows NT® or Microsoft® Windows 2000® operating system
- The Internet and Web browser terminology
- DNA sequence detection and analysis methods
- DNA and amino acid coding conventions

Conventions Used in This Guide

Text Conventions

This guide uses the following text conventions:

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

  Type **0**, then press **Enter** for each of the remaining fields.
- Titles of documents and CDs are shown in italics. For example:

  ABI PRISM® SeqScape® Software Version 2.0 User Guide
- *Italic* text indicates new or important words and is also used for emphasis.
- A right arrow bracket (>) separates successive commands you select from a drop-down or shortcut menu. For example:

  Select **File > Open Project**.

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

File Naming Convention

Some alphanumeric characters are not valid for user names or file names. The characters that are illegal are listed below:

spaces

\ / : * ?“ < > |

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 software operation.

Examples of the user attention words appear below:

Note: Names for Reference Segments are not editable.

IMPORTANT! Do not click OK until you have completed the RDG.

How to Obtain More Information

Related Documentation

The following related documents are shipped with the software:

- SeqScape Online Help – Provides procedures for common tasks. Help is available from the Help menu in the main SeqScape window, or by pressing F1.
- *ABI PRISM® SeqScape® Software Version 2.0 Tutorial*
- *ABI PRISM® SeqScape® Software Version 2.0 Quick Reference Card*

Portable document format (PDF) versions of the Applied Biosystems documents listed above are also available on the SeqScape software installation CD. If you do not have Acrobat Reader installed on your computer, install it from the SeqScape CD, so you can open the pdf files.

Note: For additional documentation, see "How to Obtain Services and Support" on page xvi.

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.

Introduction to ABI PRISM SeqScape Software

1

This chapter contains:

New Features in SeqScape Software v2.0

The following features are new in ABI PRISM® SeqScape® Software Version 2.0.

- **Extended Reference Data Group.** SeqScape Software v2.0 contains an extended reference data group (RDG). The RDG contains a known reference sequence and any known nucleotide or amino acid variants. The RDG available in this new version of the software enables analysis of simple or complex projects.

  The Reference Sequence within the RDG can be a:

  – Contiguous reference sequence with a single reading frame.
  – Contiguous reference sequence with multiple reading frames.
  – Reference sequence constructed from several reference segments. Each segment can come from different locations in the genome.

  The reference sequence can contain features such as exons, introns, splice junctions, primer-binding sites, and promoter regions.
- **Frameshift deletions.** SeqScape identifies potential instances of this variant which often require manual review by trained personnel.
- **Library searching.** You can compare each consensus sequence to a sequence library to identify the closest match genotype, allele or haplotype.
- **Enhanced reports.** You can customize reports. Each variant in the report is hyperlinked to the sequence data, providing rapid transition from results to data. The results reports eliminate the need to manually record results. You can automatically sort and reorganize any report.
- **Password protection and audit trail.** The software protects your data by providing password protection, automatic lockout when the software is inactive, and three levels of access control. An audit trail records each manual insertion, deletion, or base modification, with reasons for each change.
- **Integration automation.** The software uses an improved process for setting up samples for Applied Biosystems 3730/3730*xl* automated analysis.
- **New Basecallers.** The KB basecaller is a new algorithm that identifies mixed or pure bases and generates sample quality values. The ABI basecaller is an algorithm used in sequencing analysis software.

Updated Features in SeqScape Software v2.0

The following features are updated in SeqScape Software v2.0:

- Option to basecall with ABI basecaller only is no longer available. In SeqScape v1.1 Software, you can choose to basecall data with ABI basecaller or ABI basecaller with TraceTuner™ Software. In software v2.0, you do not have the option to basecall with ABI basecaller only. The new options are:
  - Basecall with ABI and TraceTuner (automatic)
  - Basecall with KB basecaller
- Implicit Reference is no longer available. In software v1.1, you can have an empty RDG and use the first specimen as your implicit reference sequence, but this is no longer available in v2.0. However, you can create an RDG and add an .abi sample file as a reference sequence.

About SeqScape Software

Genetic Analyzer Applications

SeqScape software is one of a suite of Applied Biosystems Genetic Analyzer software applications designed to control an instrument, collect data, and manage automated analysis. This suite of data collection and analysis software systems includes:

- ABI PRISM® GeneMapper™ Software – Performs genotyping using fragment analysis methods.
- ABI PRISM® Sequencing Analysis Software – Displays, analyzes, edits, and prints sequencing files.
- ABI PRISM® SeqScape® Software – Performs sequence comparisons for variants identification, SNP discovery, and SNP validation.

SeqScape Software Applications

Common resequencing applications include:

- SNP discovery and validation
- Mutation analysis and heterozygote identification
- Sequence confirmation for mutagenesis or clone-construct confirmation studies
- Identification of genotype, allele, and haplotype from a library of known sequences

Resequencing Data with SeqScape Software

SeqScape software allows analysis of resequenced data, comparing consensus sequences to a known reference sequence and optionally searching against a sequence library.

For example, a simple project might contain one contiguous reference sequence in a single reading frame, with no known nucleotide or amino acid variant information. SeqScape software compares a consensus sequence to this reference sequence, identifying any differences.

A more complex project might include a reference sequence constructed from several reference segments representing multiple exons and introns. You can use SeqScape to:

- Build unique sequence layers composed of different groupings of reference sequence features.
- Compare consensus sequences to each unique layer.
- Identify differences.
- Compare the sequence to a library of sequences to identify the closest match.

Data Sources for Resequencing Projects

You can create projects in SeqScape software using sequencing data generated from the following systems:

- ABI PRISM® 310 Genetic Analyzer
- ABI PRISM® 377 DNA Sequencer
- ABI PRISM® 3100-*Avant* Genetic Analyzer
- ABI PRISM® 3100 Genetic Analyzer
- ABI PRISM® 3700 DNA Analyzer
- Applied Biosystems 3730 DNA Analyzer
- Applied Biosystems 3730*xl* DNA Analyzer

Each project can contain:

- Unanalyzed sample files
- Previously basecalled sample files
- Text sequences
- Aligned consensus sequences

A single project can contain sample files from one or a mixture of instrument platforms. The software analyzes the data, displays several views of the analyzed project, and reports on results for quality control and data review.

Levels of Automated Analysis

SeqScape software performs two levels of analysis:

- It identifies variants, positions that differ from the reference sequence, and classifies those variants as known or unknown.
- It searches a library of alleles or haplotypes to identify the alleles that most closely match the sample.

What the Software Does

When you have added a reference sequence, a library, and sample files, SeqScape software performs two levels of analysis:

- Identification of nucleotide and amino acid variants.The software identifies positions that differ from the reference sequence and classifies those variants as known or unknown variants.
- Identification of genotypes, alleles, or haplotypes from a library. In addition to identification of variants, the software searches a library of genotypes, alleles, or haplotypes and identifies the alleles that most closely match each consensus sequence.

How the Software Performs Analyses

You provide the following information to the system before analysis:

- A reference sequence (backbone) made up of one or more reference segments and any known nucleotide variant information or amino acid variant information. (SeqScape software uses the backbone to classify all polymorphic positions as known variants or unknown variants.)
- An allele library (a set of sequences for the alleles or haplotypes).

Using the reference sequence, variants, allele library, and software settings, you create a reusable project template. With this template and the sequencing samples, SeqScape software:

- Performs basecalling, quality value assignment, and mixed base identification, in that order.
- Trims low-quality bases from each sequence.
- Identifies poor-quality samples and removes them from further analysis.
- Assembles the remaining samples against the reference sequence and generates a specimen consensus sequence.
- Reviews the basecalling quality values and the sample assembly to confirm, improve, and assign quality values to the consensus sequence.
- Identifies variants by aligning specimen sequences to the reference sequence and comparing the specimen consensus sequences to the reference sequence.
- Generates nine detailed reports.

Note: If you link a library to a project, the software also automatically searches the library to find the closest match to each consensus sequence.

When the analysis is complete, the software generates a project file that contains sample files, a consensus sequence for each specimen, and nine reports. You can print and export your results.

Getting Started 2

This chapter contains:

Administrator: Registering the Software

This chapter provides information you need to know before installing and using the ABI PRISM® SeqScape® Software Version 2.0. The administrator must follow the procedures in this section, "Administrator: Registering the Software," through "Starting the SeqScape Software for the First Time" on page 2-9.

License and Warranty

Before you begin, read Appendix F, "Software Warranty Information." Appendix F explains your rights and responsibilities regarding the SeqScape software. During the installation process of the software, you must accept the terms and conditions of the Software License Agreement before the software can be installed.

Registering Your Software

To register your copy of the SeqScape software, complete the registration card (included in this software package) and return it to Applied Biosystems.

Registering the software enables Applied Biosystems to send you notification of software updates and any other future information that may be specific to SeqScape software owners.

IMPORTANT! Your product registration number is located on the registration card. Be sure to record the number here before you return the registration card.

Registration Number:

Hardware and Software Requirements

The SeqScape software can be installed on a computer, provided it meets the minimum requirements stated below.

Minimum System Requirements

Table 2-1 summarizes the minimum system requirements for running the SeqScape Software v2.0 for Windows NT® or Windows® 2000 platforms on your instrument or analysis computer.

Note: In general, the more memory, the larger the screen size, and the more processing power in the system, the better its performance.

Table 2-1 Minimum System Requirements

System Component	Minimum Requirements
CPU	733 MHz or faster with an Intel Pentium® III or IV processor. The software does not run on computers with a dual processor or with a Xeon chip set.
CD-ROM drive	Any
Operating system	Microsoft® Windows NT® v. 4.0 OS with Service Pack 5 or Microsoft® Windows 2000® OS with Service Pack 2.
RAM	256 MB. Applied Biosystems recommends 512 MB.
Printer	An HP® 4500, 8100, 990cxi or an Epson® 980 printer is recommended.
Monitor	A 17-inch monitor or larger is recommended. A monitor of 1024 × 768 resolution is recommended.

Table 2-1 Minimum System Requirements *(continued)*

System Component	Minimum Requirements
Disk space	1 GB. Storage requirements depend primarily on the quantity of data to be generated and stored. It is common to store many SeqScape software project files on the analysis computer. Because SeqScape software stores data files in the area where the program is installed, you should install SeqScape software on a partition with enough space for the projects and their files.

Hard Drive Partitions

The installer uses the following location for the SeqScape software files:

drive letter:\Applied Biosystems\SeqScape

The drive letter is determined by the following conditions:

Table 2-2 Drive Letter Conditions:

If the computer …	The installer selects drive …
is not connected to a genetic analyzer	D (default) C (if D drive is not available)
has Data Collection software that is connected to the Applied Biosystems 3730/3730*xl* DNA Analyzers	E

Installing the SeqScape Software

The SeqScape v2.0 software can be installed in one of two ways:

- Install on a computer with no previous version of SeqScape software
- Upgrade a previous version of SeqScape software (v1.0 or v1.1)

Before Installation

An administrator should install the software and use it for the first time. The administrator can set up the software for the analyst, scientist, or other administrator users.

To prepare for the installation:

1. Ensure that your system meets the minimum requirements (see "Hardware and Software Requirements" on page 2-3).

   Check that you have at least 1 GB of free disk space to accommodate the SeqScape software, and sufficient space for all projects and their sample files.

2. If you use data stored in a database, verify that the computer has TCP/IP installed.

3. Exit all programs except Applied Biosystems 3730 Data Collection software, if applicable.

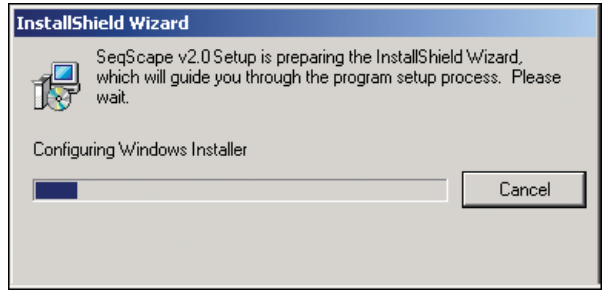
IMPORTANT! To properly install SeqScape software v2.0 on a computer that is connected to a 3730/3730*xl* DNA Analyzer, the data collection software must be running. If data collection is not running, the SeqScape software does not register with the Data Service. See Chapter 11, "Automating Analysis," for more information on file sharing and automation.

Installing for the First Time

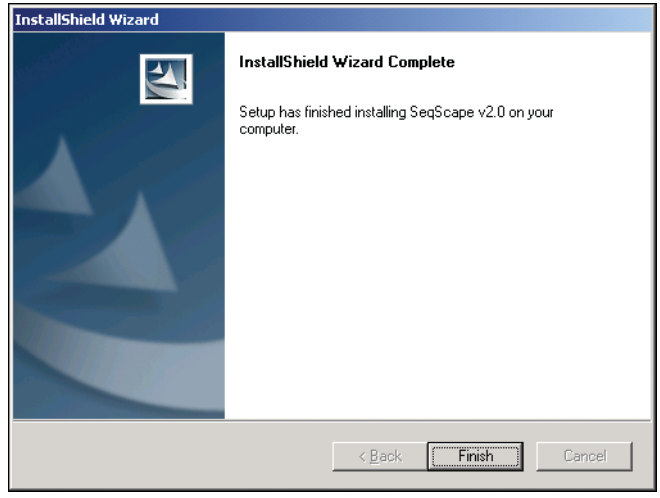
This section gives instructions to install SeqScape Software v2.0 for the first time onto a computer that does not have a previous version of the software. The administrator of the software installs the software and sets up new users.

To install the SeqScape software for the first time:

1. Insert the *ABI PRISM® SeqScape™ Software v2.0* CD into the computer CD-ROM drive.
2. If the installer does not start automatically, double-click **setup.exe** on the CD.
3. Follow the instructions to install the software.



4. When the InstallShield Wizard Complete window opens, click **Finish**.



After the software is installed, the administrator must log into the software for the first time. After the initial login, the software can be set up for additional users.

Upgrading from SeqScape Software v1.0 or v1.1

About the Upgrade

If you are upgrading from a previous version of software to SeqScape software v2.0, the installer automatically uninstalls a previous version of the software when it installs a newer version. Follow the instructions on the installer.

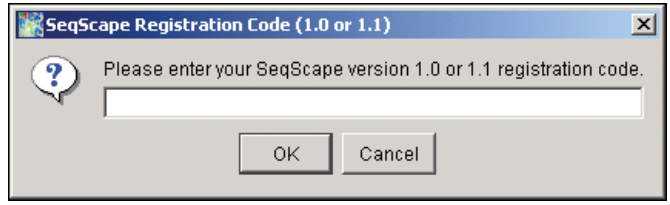
The SeqScape Software v2.0 installer:

- Detects the previous version and backs up your data folder
- Removes the previous version, then installs the new version of SeqScape software

Upgrading to v2.0

To upgrade your software:

1. Insert the *ABI PRISM® SeqScape Software v2.0* CD into the computer CD-ROM drive.
2. If the installer does not start automatically, double-click **setup.exe**.
3. When the following dialog box opens, enter your registration code for v1.0 or v1.1.



4. Follow the instructions to upgrade the software.

After the software is installed, you must register and log in as an Administrator user. After you log in as Administrator, you can set up additional users with Admin, Scientist, or Analyst permissions.

Existing Users

All existing users of an earlier version of SeqScape software will have Analyst privileges. Only a user belonging to the Administrator group can change the user to Scientist or Analyst. A dialog box opens for users who existed in previous versions to set up their user profiles (name and password) when they try to use SeqScape Software v2.0 for the first time.

Removing SeqScape Software v1.0 or v1.1

What the Uninstallation Process Does

To completely remove the SeqScape software from your computer, follow the procedure in this section. The uninstallation process:

- Deletes all folders and files installed by the SeqScape software. However, if you moved the SeqScape Software folders or files from their original installed location, they may not be found and deleted by the uninstallation process.
- Does not delete any files or folders created by users. Any files that have been added to the application folders, such as those created when the applications are run, are not deleted by the uninstallation process.

To uninstall the SeqScape software:

1. Select **Start** > **Programs** > **Applied Biosystems** > **SeqScape** > **Uninstall SeqScape v1.0 or v1.1**.
2. Continue to follow the instructions to uninstall the software.

   When the uninstallation is complete, all the software program files are removed. Your data files remain on the computer. The uninstaller does not delete any folders or files created after installation. If you want to delete any folders and files created after installation, you must remove them manually.

Starting the SeqScape Software for the First Time

Before You Begin

The SeqScape software is designed with a user login process. When you start the software for the first time, you are prompted with a registration dialog box that creates an administrator account. Log in to the SeqScape software as Admin and enter the password you created.

To create new users, you must log in as Admin. Logging in with a user name allows SeqScape software to track each user's interactions with each project.

For information on the privileges for each category of user using the software, refer to Appendix D, "User Privileges."

File-Naming Convention

Some alphanumeric characters are not valid for user names or file names. The invalid characters are below:

spaces

\ / : * ? " < > |

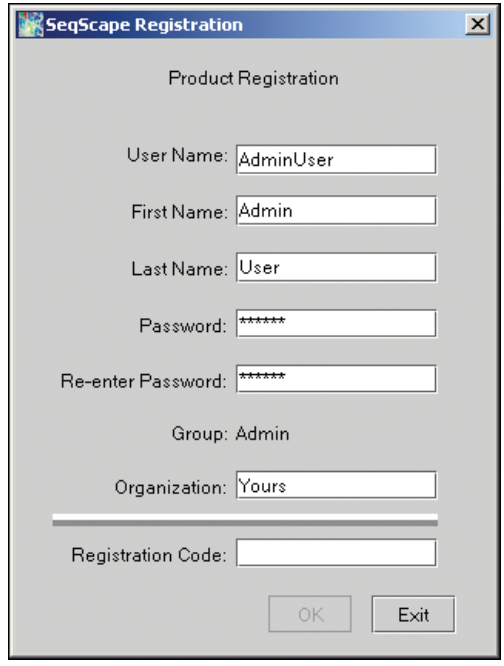
An error message is displayed if you use any of these characters. You must remove the invalid character to continue.

IMPORTANT! User names cannot be named seqscape_admin in this version of the software. If you have used this user name in a previous version of the software, you must change the user name to follow the File Naming Convention shown above.

Starting SeqScape Software

To start the software for the first time:

1. Double-click the SeqScape desktop shortcut.
2. In the **SeqScape Registration** dialog box, enter all the information in the text fields. The User Name and password must be 6 to 15 characters long.



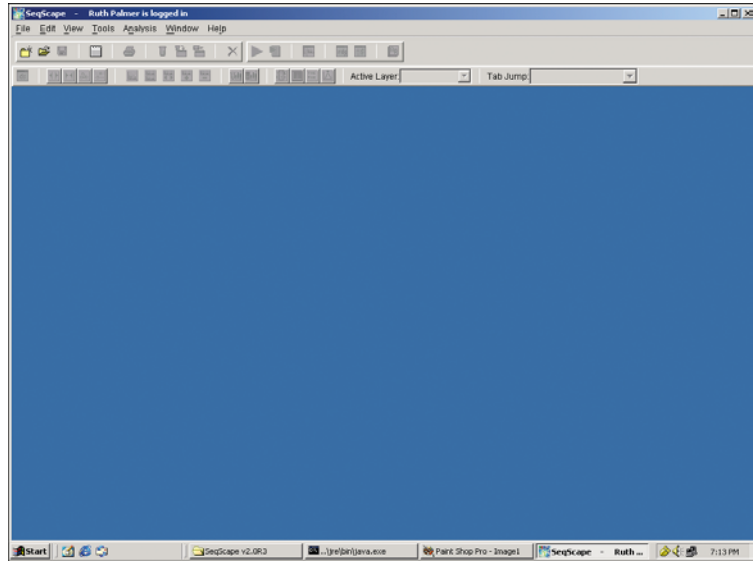
The first user created is automatically assigned Administrator privileges.

3. Enter the registration code on the registration card you received with your software.
4. Click **OK**.

   While the program is loading, the splash screen appears. When the program is finished loading, the Login dialog box opens.
5. Enter your user name and password again.

6. Click **OK**.

   The main SeqScape window opens.



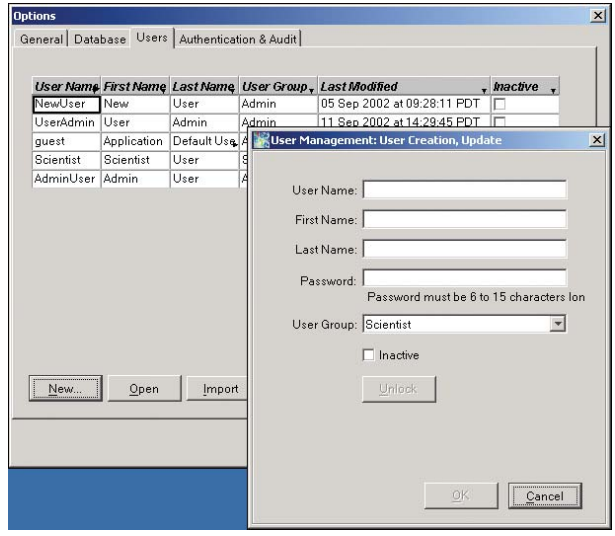
Creating New Users

Because the SeqScape software tracks the projects and settings for each user, Applied Biosystems recommends that you create users for each individual who uses SeqScape software on the computer. The Users tab allows exporting of user names and access privileges for these users.

IMPORTANT! The administrator is the only person who can set up and change the information in the Users tab. The selections in this tab are inactive for all other users.

To set up new users:

1. Select **Tools** > **Options** to open the Options dialog box.
2. In the Options dialog box, select the **Users** tab, then click **New**.



3. Fill in the appropriate user name, password, first and last names, then select the level of user from the Group drop-down list.

   Note: Enter a User Name that contains only alphanumeric characters. This field must not contain any spaces or characters that do not conform with the Microsoft® Windows file system. Refer to "File-Naming Convention" on page 2-9.

   The new user appears in the list in the Users tab.

New users can log in after you exit SeqScape software and restart the application.

Setting Up Authentication & Audit

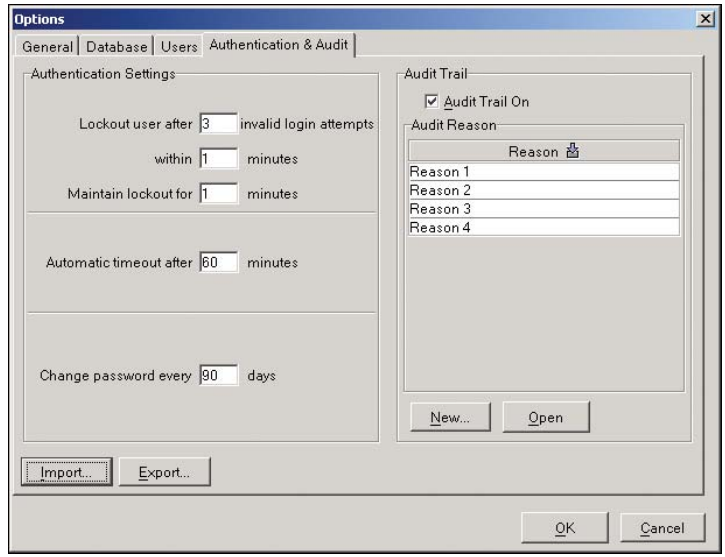
Users belonging to the Administrator group can change the default settings in the Authentication & Audit tab for security features of the application.

Note: The Administrator is the only person who can set up and change the information in the Authentication & Audit tab. The selections in this tab are inactive for all other users.

The Authentication & Audit panes provide a way to track the changes in projects such as base change, variants, or processes you want to track. You must turn Audit Trail On for tracking to occur.

To set up authentication and auditing:

1. Select the **Authentication & Audit** tab to change the defaults for the Authentication Settings:



   a. Lockout occurs when a user enters an incorrect password or user name the number of times you select for the **Lockout user after invalid login attempts** field. Enter the number or accept the default.

   b. The **within minutes** field indicates that the user will be locked out if the maximum number of attempts occur within the time entered in this field. Enter a number or accept the default.

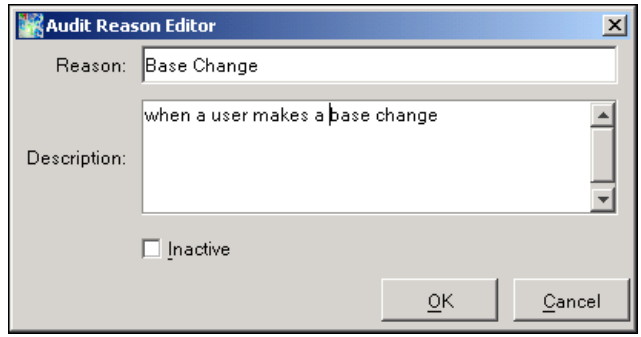
   c. The **Maintain lockout for minutes** field indicates the number of minutes that must elapse before the user can login again after being locked out of the SeqScape software. Enter the number of minutes or accept the defaults.

   d. The **Change password every days** field indicates the number of days before the users must enter a new password. Enter a number of days or accept the default.

2. In the Audit Trail pane, select the **Audit Trail On** check box to have a dialog box open whenever an indicated reason occurs.

3. In the Audit Reason pane, enter reasons to provide an audit trail.

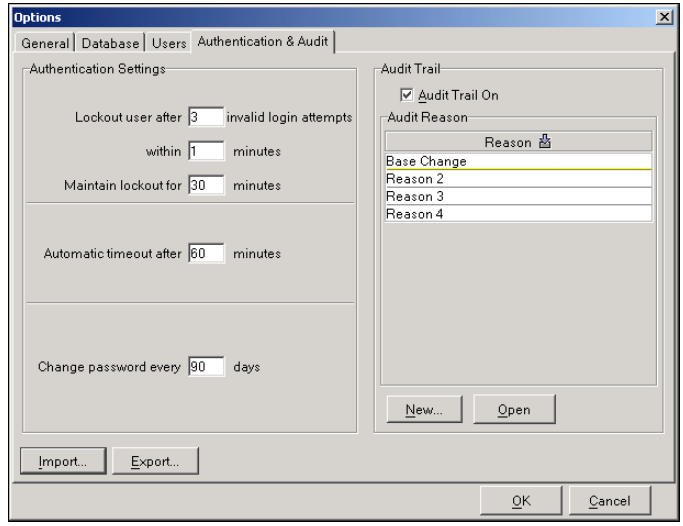
a. Double-click the **Reason 1** field, or highlight it and click **New**.



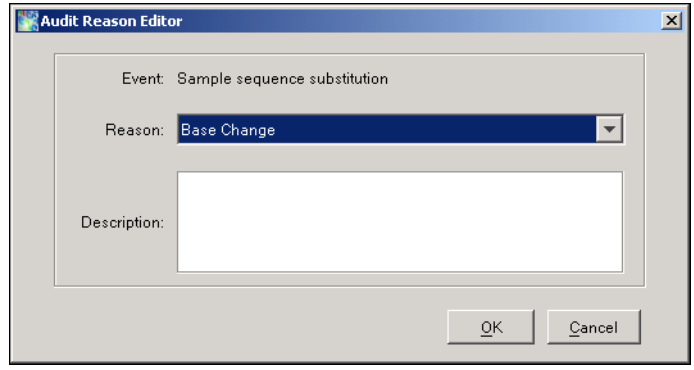
b. In the Reason field, type a reason for a change to the project to identify, for example, a base change, or a variant that is imported.

c. Enter a description of the reason, if desired.

d. Click **OK** in the Audit Reason Editor. The first reason appears in the list in the Options dialog box.



e. Whenever a change is made in any of the project views, the Audit Reason Editor dialog box opens as shown below. Select the reason for the change from the drop-down list.



4. If desired, click **Export** in the Options General tab and navigate to export the configuration settings to another computer. The Import button allows configuration settings to be imported from another computer.

5. Click **OK** in the Options dialog box to save the authentication and audit settings.

Note: It is possible to import or export Authentication & Audit configurations from one computer to another. For example, an administrator may want to set up authentication and audit information for many users, then select all the files and export them to other systems using SeqScape software.

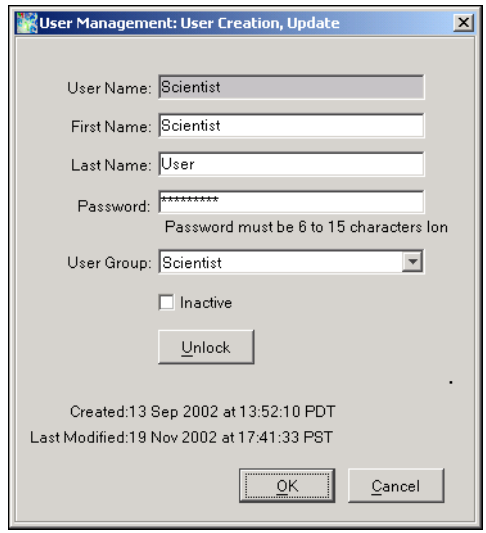
Changing User Information

If desired, change the default settings for all users you are setting up.

IMPORTANT! The Administrator is the only person who can set up and change the information in the Users tab. The selections in this tab are inactive for all other users.

To change any of the information for a user:

1. In the Options dialog box, select the **Users** tab.
2. Double-click the name in the list to open the User Management dialog box.



3. Change or correct the user information and click **OK**.
4. If desired, click the **Export** button in the Options dialog box to export the application configuration settings and/or settings for a single user or multiple users in a zipped .ctf format.
5. Enter the path for exporting files in the Export User dialog box, then click **Export**.
6. Click **OK** to close the Options dialog box.

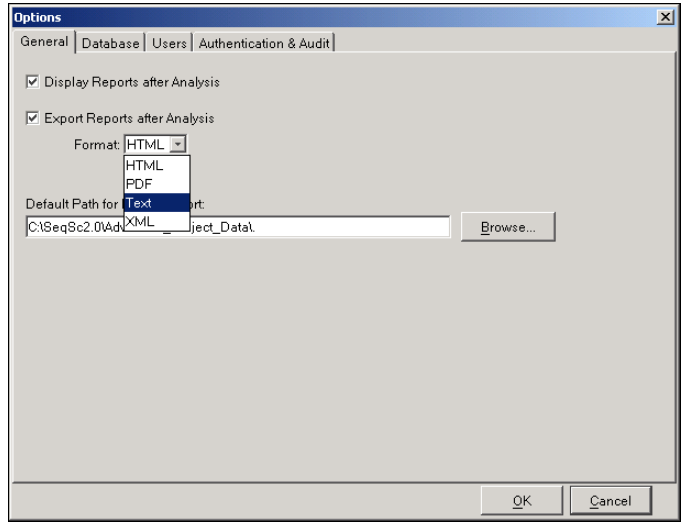
Note: This process can be used by the first administrator to set up additional users or another administrator. It is possible to import or export user settings from one computer to another. For example, an administrator can set up user information for many users, then select all the user files and export them to other systems using SeqScape software.

Setting Up the Default Directory

The default directory should be set up for users for importing and exporting data files. If the directory path is not set up, the default directory opens to C:\.

To set up the default directory path:

1. In the SeqScape main window, select **Tools** > **Options**.
2. In the General tab, select the appropriate check boxes for your setup, if desired.
   a. Select the **Display Reports after Analysis** check box.
   b. Select the **Export Reports after Analysis** check box, if desired, then select the format in which to export them from the Format drop-down list.



3. Click **Browse** and navigate to the directory to use as the default for files to be stored.
4. Click **Open**.

   The exported reports are stored in the directory you select as the default.
5. Click **OK** to save the directory path and close the dialog box.

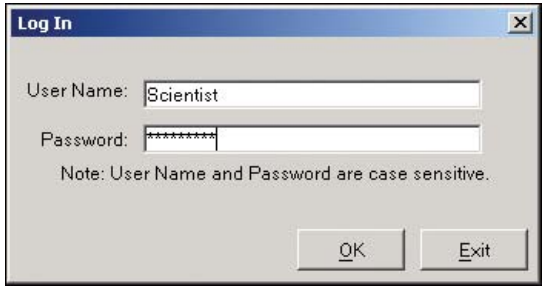
New Users Logging In for the First Time

When New Users Log In

After the installation and setup are complete, new users can log in to the software.

To log in to the software:

1. Start the SeqScape software by double-clicking the desktop shortcut  .
2. The Log In dialog box opens, showing the last user's name. Enter your user name and password, then click **OK**.



The SeqScape software is ready for you to use.

Note: All existing users of an earlier version of SeqScape software will have Analyst privileges. Only a user belonging to the Administrator group can change the user to Scientist or Analyst. Users who existed in previous versions will be asked to set up their user profiles (name and password) when they try to use SeqScape Software v2.0 for the first time.

Connecting to a Database

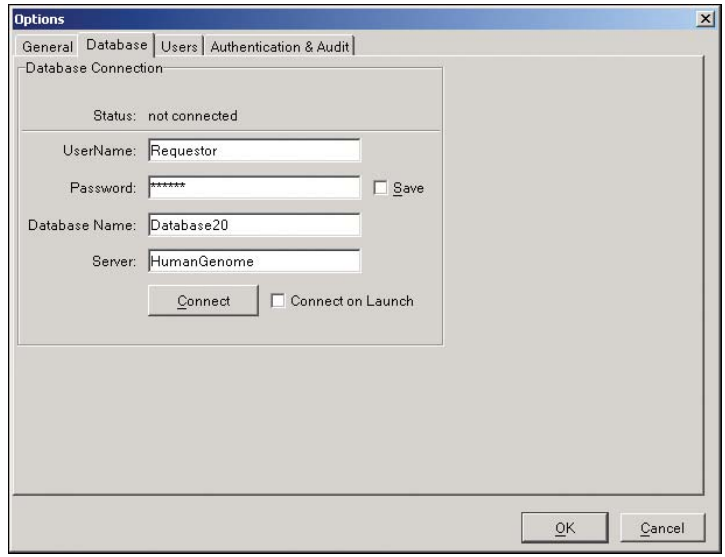
SeqScape software allows you to connect directly to a Sequence Collector v3.0 database if you have Oracle 8i client (standard edition, version 8.1.7) installed on your computer.

Note: If you have Sequence Collector v3.0 installed on your computer, you also have Oracle 8i client installed, because it is required to run Sequence Collector software v3.0.

If the Sequence Collector v3.0 database is installed on another computer on your network, you may need to perform the Sequence Collector Client installation.

To connect to a Sequence Collector v3.0 database:

1. Launch SeqScape software, select **Tools** > **Options**, then select the **Database** tab.



2. Enter the appropriate information in the UserName, Password, Database Name, and Server fields.

   Note: These names are the same as those used for Sequence Collector. See *Applied Biosystems Sequence Collector Software 3.0 User Guide* for more information.

3. Click **Connect** to start the connection.

   When a connection is made, the Status displays “connected” and a Disconnect button appears.

4. If you want to connect when you launch SeqScape software, click **Connect on Launch**, then click **OK**.

   Note: The connection is made automatically whenever you launch the SeqScape software.

   Note: If you have problems connecting to the database, contact your system administrator.

SeqScape Software Structure

The SeqScape software is organized around two main windows:

- SeqScape Manager window, from which you enter and manage the information necessary to perform analyses
- Project window, from which you manage the results of analyses

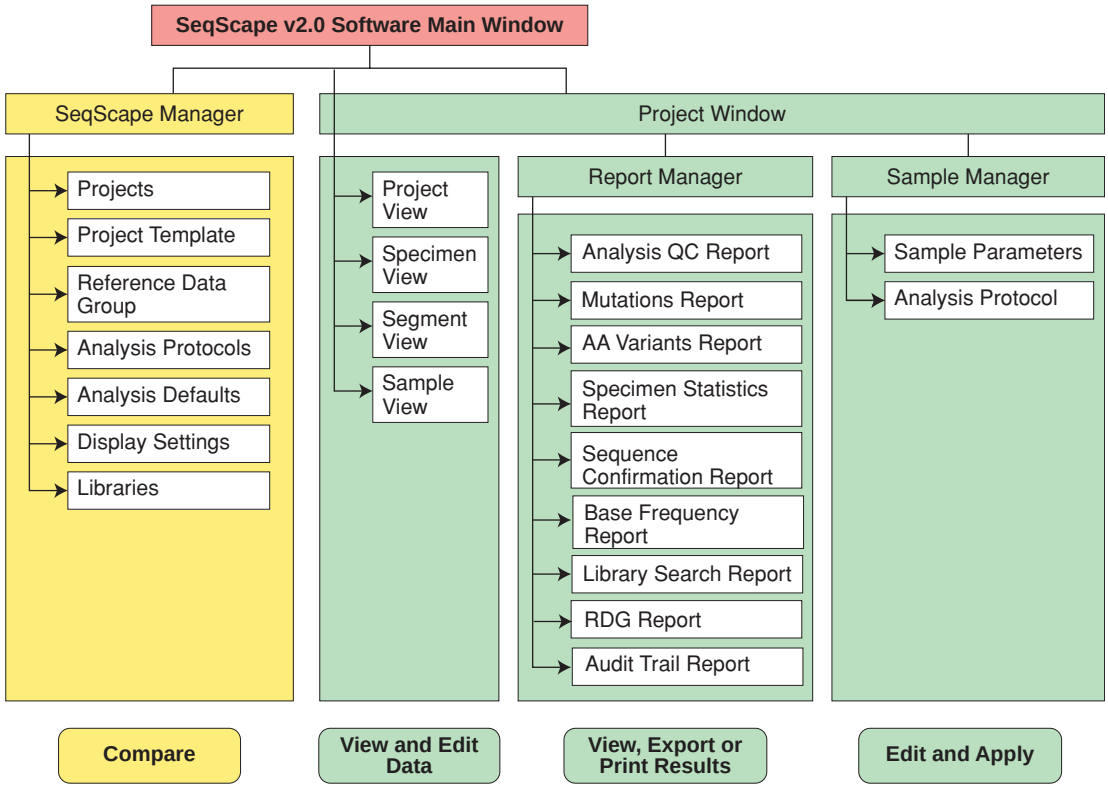


Figure 2-1 SeqScape Software Structure

SeqScape Manager Window

In the SeqScape Manager, you configure projects by creating project templates. The project templates can be reused in multiple projects and can be exported to be shared with other researchers. The project templates contain:

- Reference sequence information
- Analysis settings (including analysis protocols)
- Display settings

Project Window

In the Project window, you can view your data in the following ways:

Table 2-3 Views in the Project Window

View	Description
Project View	Shows the reference sequence, each specimen consensus sequence, and electropherogram snippets for each sample file in each specimen. The Expanded Nucleotide View shows all the nucleotides. The Collapsed Nucleotide View shows only variants of the nucleotides. The Expanded Amino Acid View shows all the amino acids. Characters (NT or AA) that are the same as the reference are shown as dots. The Character/Dots button switches to show or hide the view. The Identification pane, which shows the library search results, appears at the bottom of the Project view.
Specimen View	Shows the clear range and orientation of each sample and how they line up to the reference sequence, and the overview pane with active ROIs.
Segment View	A table of sample information. Clicking a row in the table shows the corresponding sample sequence below. The Layout tab shows the direction of each sample within the segment. The Assembly tab shows samples aligned to the consensus sequence. An overview pane that represents forward and reverse sequences, variants, and ROIs. Electropherograms can be displayed for one or all sequences.
Sample View	Shows pertinent information for the sample, which includes annotation, sequence, electropherogram and raw data.

Refer to Chapter 10, “Sample and Consensus Quality Values,” for detailed descriptions of these views.

SeqScape Software Toolbar

The SeqScape software toolbar displays buttons for software functions that you are likely to use often. Refer to the next two figures for the names, descriptions, and keyboard shortcuts for each button. The top row of buttons, Figure 2-2, are processing tools.

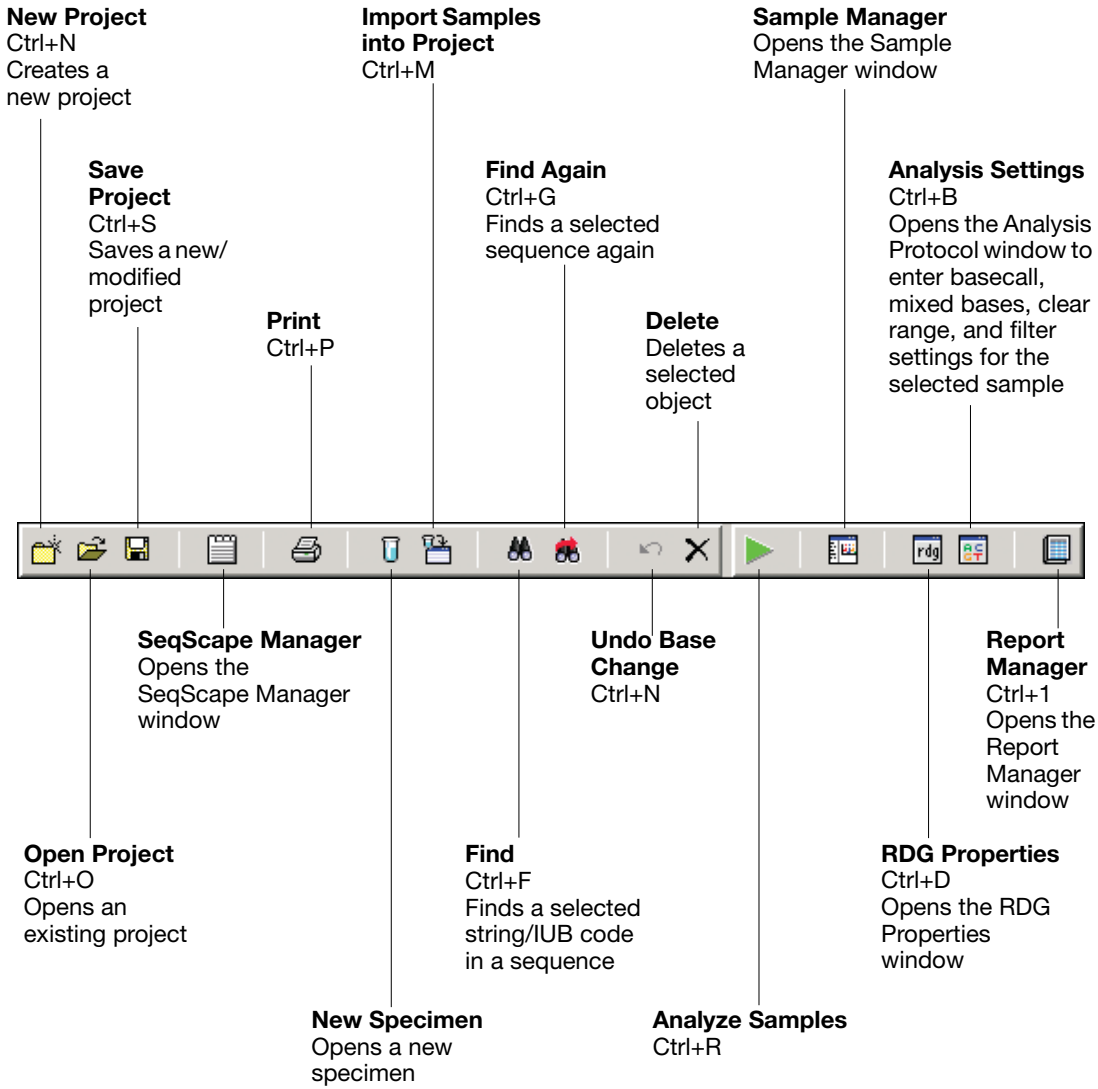


Figure 2-2 Main Toolbar

The second row of buttons, Figure 2-3, are viewing options for the projects you create.

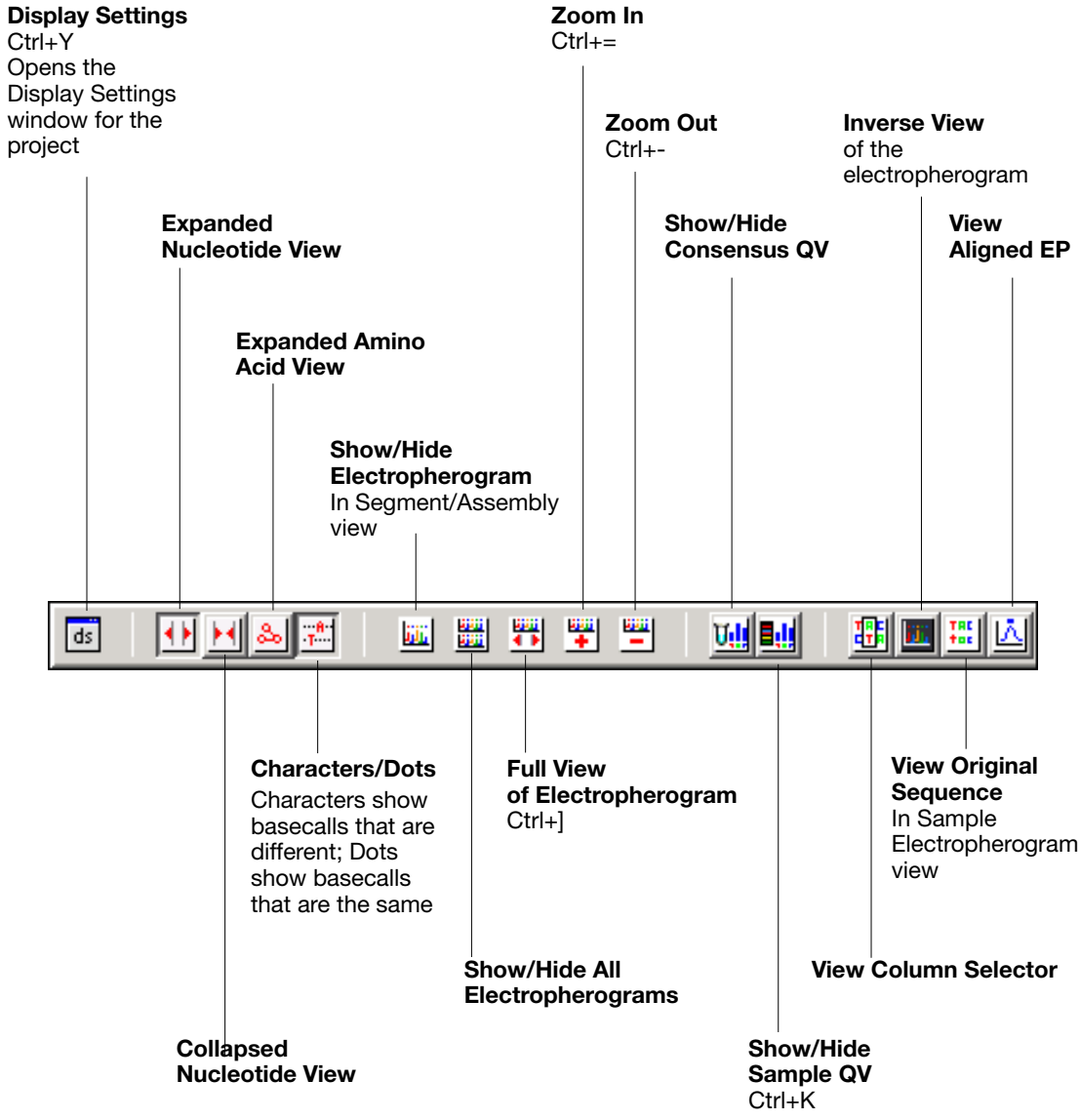


Figure 2-3 Viewing Toolbar

Menus on the Main SeqScape Window

Figure 2-4 shows the menu structure of the main SeqScape window.

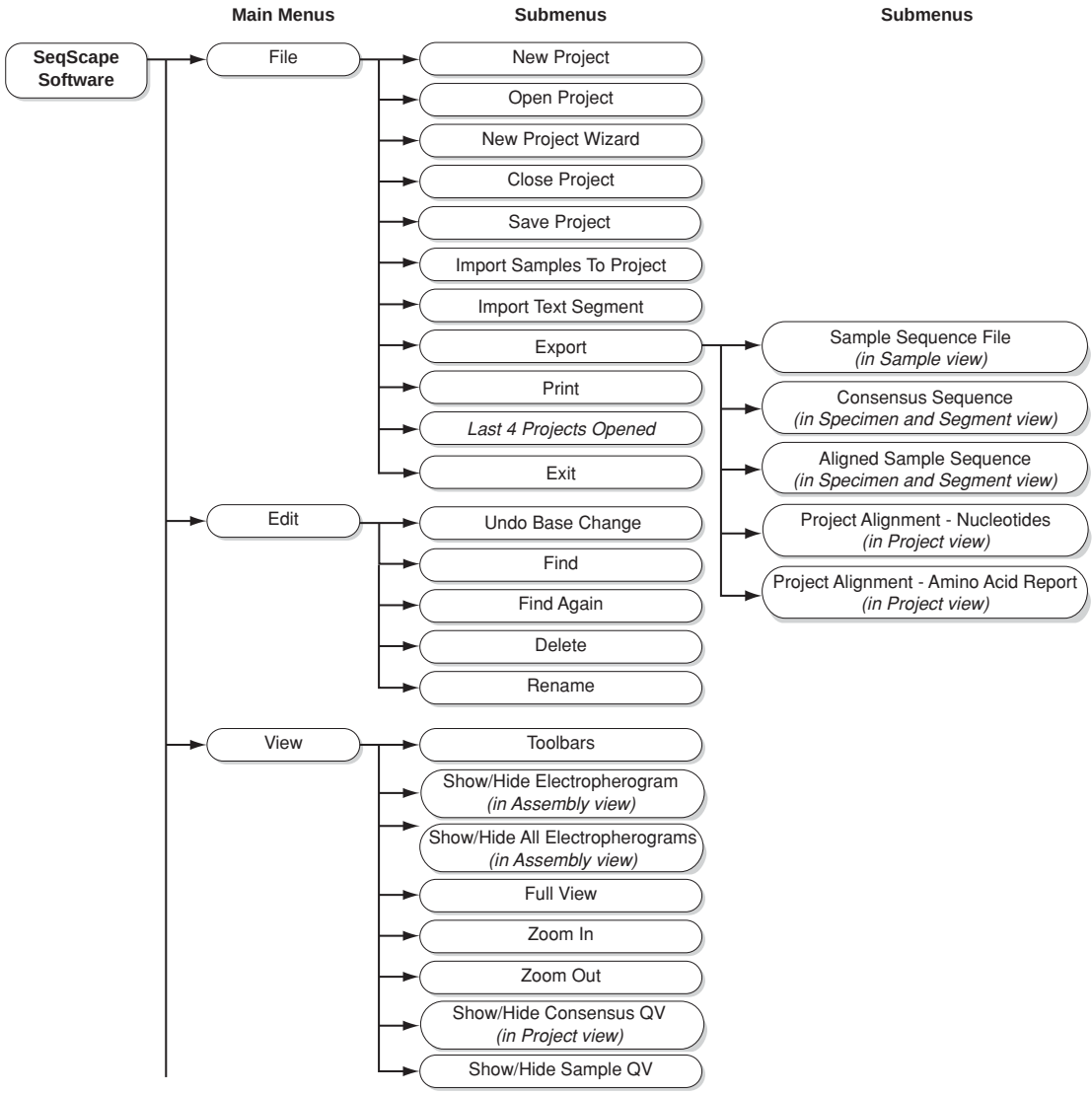


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Figure 2-4 Main SeqScape Window Menus

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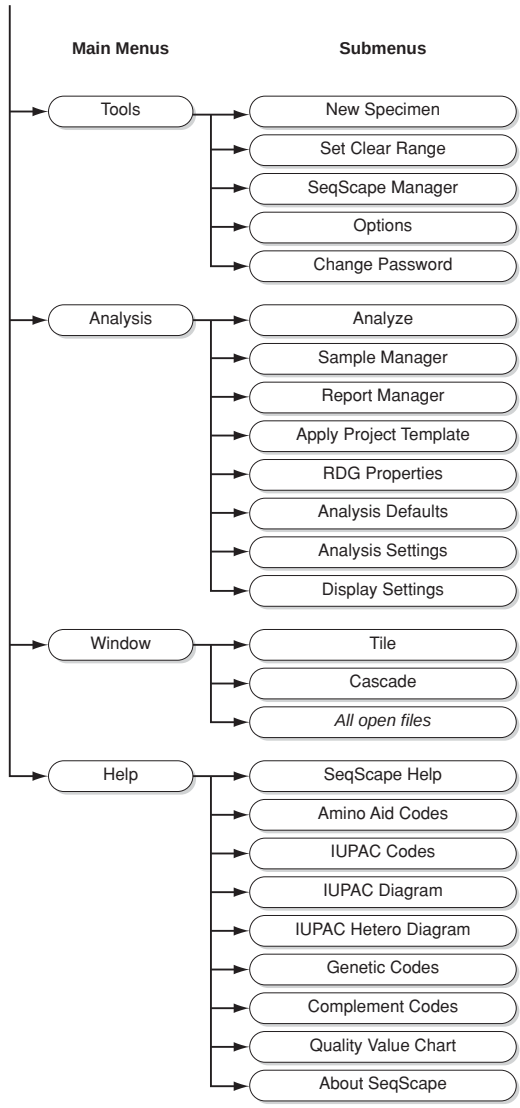


Workflow

A typical workflow using the SeqScape software is shown below.

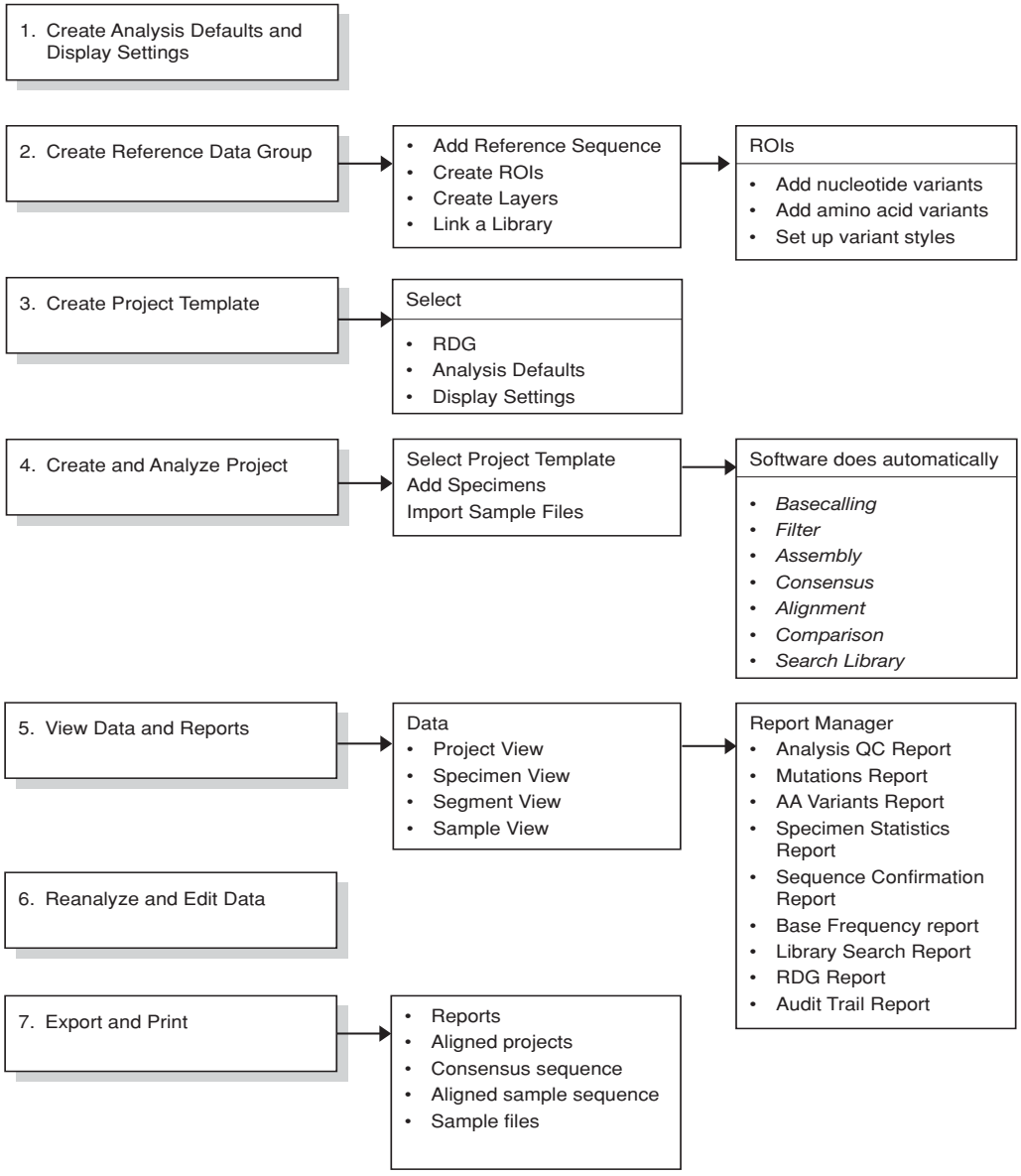


Figure 2-5 Typical Workflow for a Project

Creating Analysis Defaults and Display Settings

3

This chapter contains:

Workflow for This Chapter

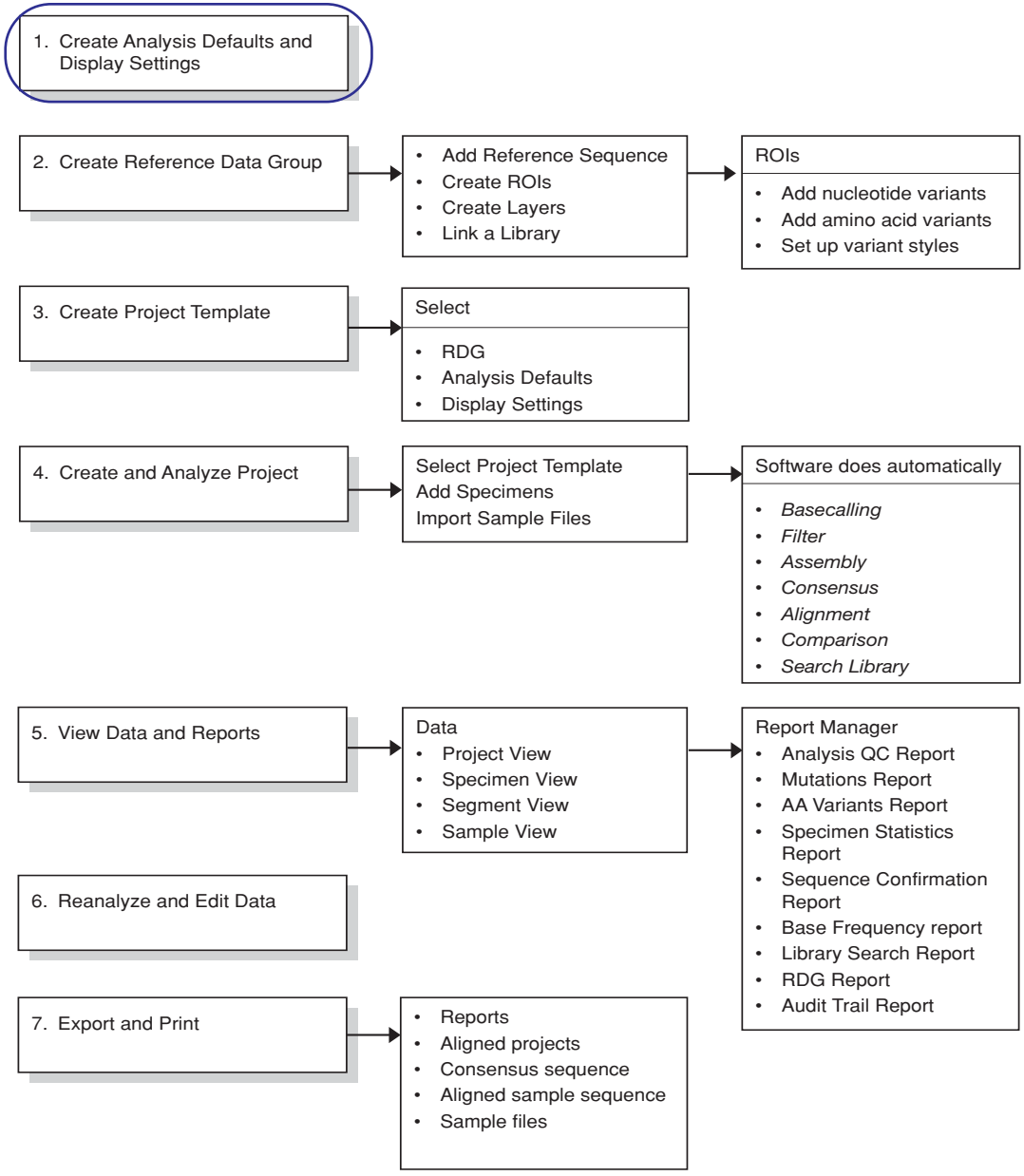


Figure 3-1 Create Analysis Defaults and Display Settings Step

Analysis Defaults Settings

Analysis Defaults are a component of the project template. They are used to set the analysis settings for all the samples as they are imported into a project.

Creating Analysis Protocols

Before you create analysis defaults, you need to create the analysis protocol. An analysis protocol in the ABI PRISM® SeqScape® Software Version 2.0 specifies the analysis conditions to be applied to your samples. You can specify the analysis protocol settings for one or more samples. You must select an analysis protocol before selecting analysis defaults. The protocol settings include:

- Basecalling
- Mixed bases
- Clear range
- Filtering

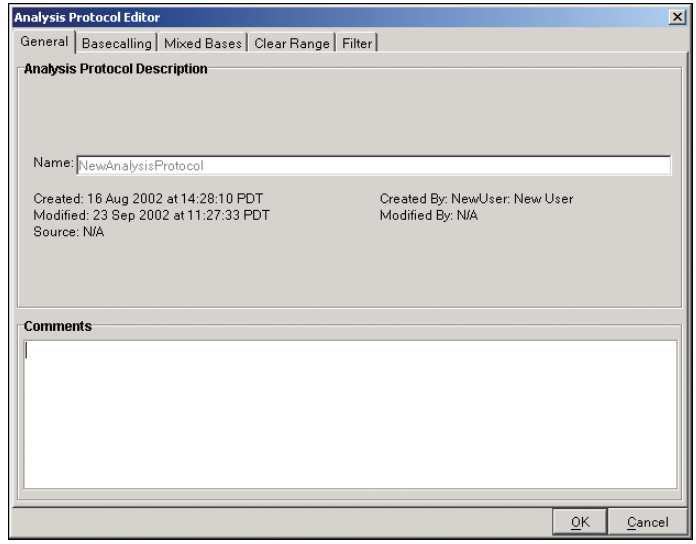


Figure 3-2 Analysis Protocol Editor Showing General Tab

Analysis Protocol Editor Tabs

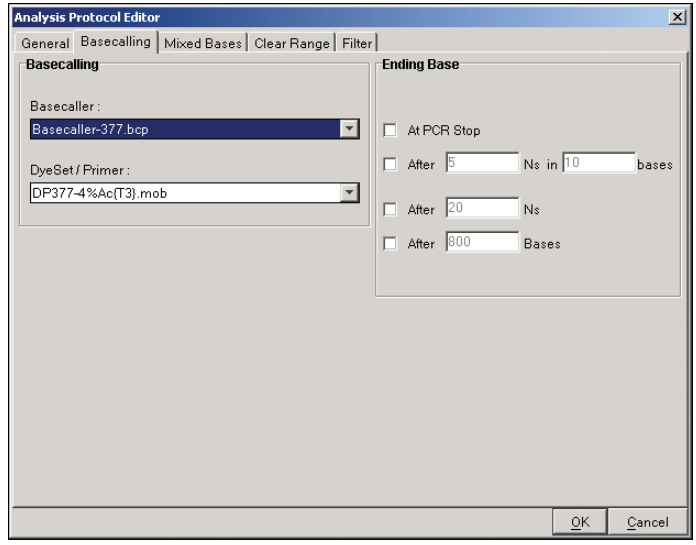
The Analysis Protocol Editor tabs and descriptions are used to set up the analysis protocol to select with the analysis defaults.

- The **General** tab contains general information on the analysis protocol, for example, the name, creation date, and modification date. Refer to Figure 3-2.
- The **Basecalling** tab has settings for how the software calls bases. The basecaller you select is determined by the instrument and chemistry you are using. For further details on basecalling files and dye primer set selections, see Appendix A, "Basecallers and DyeSet/Primer Files."
- In the **Mixed Bases** tab, the Use Mixed Bases Identification box generates calls following the international standard IUB code for heterozygous positions. Mixed bases identification occurs only if secondary peak threshold is equal to or more than a specified percentage of the highest peak. You set the level according to sample type, reaction kit, and purification reaction.
- **Clear Range** is the region of the sequence that remains after excluding the low-quality or error-prone sequence at the 5′ and 3′ ends. You can specify a range as a default. It is recommended that you always check Use reference trimming.
- The **Filter** tab sets the criteria for rejecting sequences if they do not meet minimum standards. Sequences not meeting the filter settings are not assembled.

Specifying the Basecall Settings

To specify the basecall settings:

1. Select **Tools** > **SeqScape Manager**.
2. Select the **Analysis Protocols** tab, then select the project in the list for which you want to change the settings.
3. Click **Properties**.
4. In the Analysis Protocol Editor, select the **Basecalling** tab to view the basecalling settings.
5. Select the appropriate basecaller algorithm dedicated to your instrument. For more information, refer to Appendix A, "Basecallers and DyeSet/Primer Files."



6. Select the DyeSet/Primer settings (mobility files, .mob extension) for the instrument you are using. For more information, refer to Appendix A, “Basecallers and DyeSet/Primer Files.”

7. If you have short PCR products, you should end basecalling at the end of the PCR product. In this case, select the **At PCR Stop** check box.

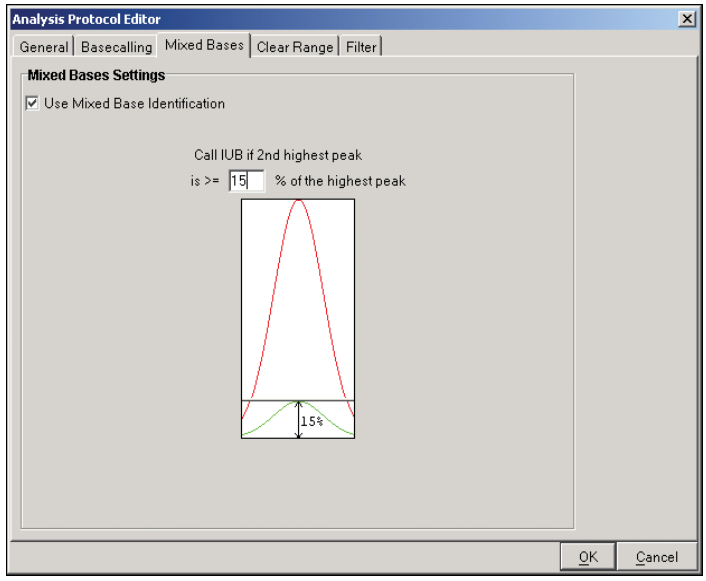
8. You can also stop basecalling after a specified number of ambiguities, or Ns, or after a certain number of bases. Enter your changes to the settings.

   For more information on basecaller settings, refer to the *ABI PRISM® DNA Sequencing Analysis Software User Guide.*

Specifying the Mixed Bases Settings

To specify the mixed bases settings:

1. In the Analysis Protocol Editor, select the **Mixed Bases** tab.



2. Select the **Use Mixed Base Identification** check box to generate calls according to the international standard IUB code for heterozygous positions. Mixed bases identification occurs only if the second peak height is greater than or equal to a percentage of the main peak height.

3. Set the level according to sample type, reaction kit and purification reaction, and expected or acceptable percentage. Enter the threshold for calling a mixed base for the % value of the primary peak.

   IMPORTANT! If you decrease the default percentage to detect low-percentage mixed bases, the background signal may be higher and interfere with mixed base detection. Be aware of this condition.

Specifying Clear Range

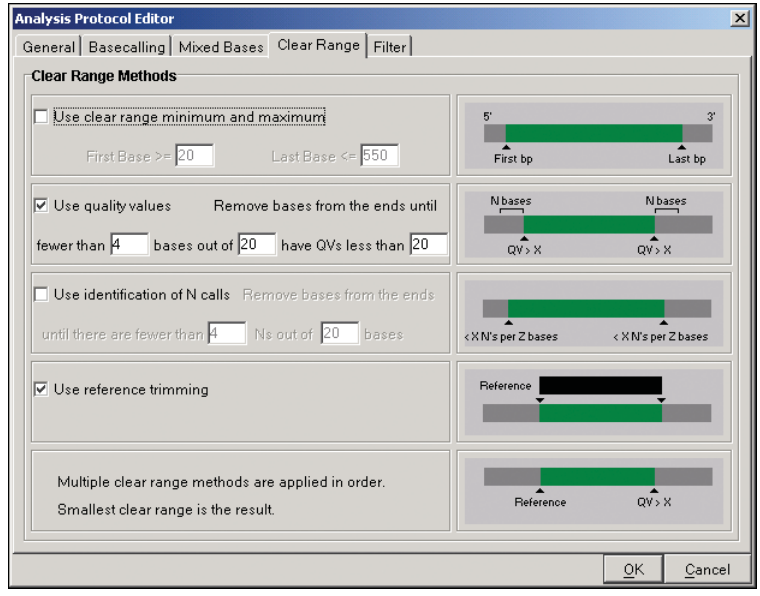
You can apply all or a subset of the Clear Range Methods algorithms. Each is applied in order from top to bottom, with the clear range method never being lengthened based on the settings in subsequent algorithms. The result is that the smallest clear range is used. If you want to preserve the existing clear range in a sample when reapplying analysis protocol settings to a sample, do not select any of the Clear Range methods.

IMPORTANT! You can create a protocol without selecting a clear range method, but it is recommended that at least one clear range method be selected for reference trimming.

The Clear Range tab enables you to set the part of the sequence that you consider to be good quality. Good quality means that the sequence has the fewest errors and ambiguities, and offers good base calling and spacing.

To set the way the clear range is determined:

1. In the Analysis Protocol Editor, select the **Clear Range** tab.



Because SeqScape software generates quality values for each base, you can choose to use a region of sequence where a certain number of bases reach an appropriate quality value.

2. Select **Use clear range minimum and maximum,** then set the minimum first base and maximum last base of the clear range.

3. Select **Use quality values** to remove bases until there are < X number of bases per Z number of bases with QV < Y. This sets a window with a specified number of allowed low-quality bases.
4. Select **Use identification of N calls** to remove bases until there are < X number of Ns per Y number of bases. This sets a window with a specified number of allowed ambiguous base calls (Ns).
5. Select **Use reference trimming** to have the samples automatically trimmed to contain only sequences that align to the reference.

Specifying the Filter Settings

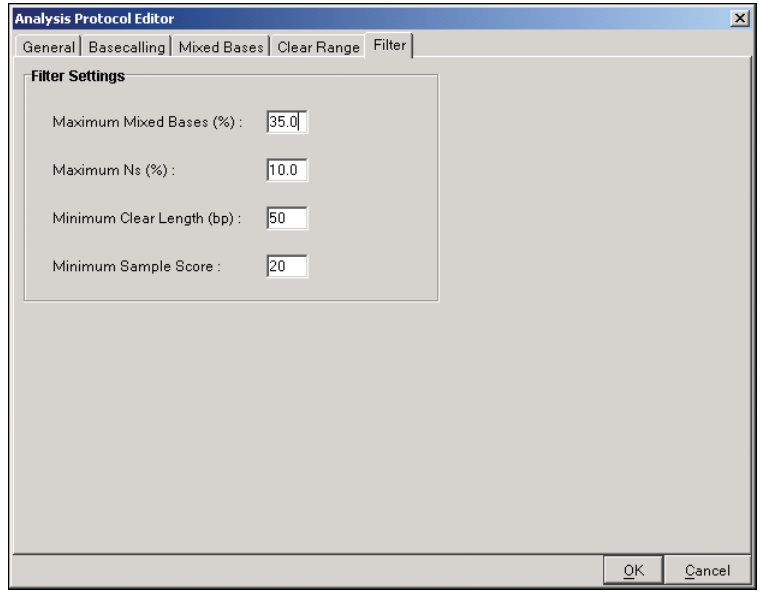
The Filter tab sets the criteria for rejecting sequences if they do not meet minimum standards. Sequences not meeting the filter settings are not assembled.

Use the maximum percentage of mixed bases to look for frame shift. Use the maximum percentage of ambiguities (N) and the minimum length settings to ensure that you are working with enough data for further analysis. This filters data that may exceed a specified percentage of ambiguities.

Also, use a minimum sample score to ensure that the quality of the sequences is high. A setting of 20 indicates that the data is accepted if the mean quality value of all bases in the clear range is 20 or greater. This corresponds to a 1-to-100, or 1%, error rate.

To select the filter settings:

1. In the Analysis Protocol Editor, select the **Filter** tab.



2. Enter your changes to the settings using the descriptions of the settings in the following table as a guide.

Parameter	Description
Maximum Mixed Bases (%)	Total maximum percentage of mixed bases that can occur in the clear range of a sample file. Any more than this number causes the sample to fail analysis.
Maximum Ns (%)	Total maximum percentage of Ns that can occur in the clear range of a sample file. Any more than this number causes the sample to fail analysis.
Minimum Clear Length (bp)	Minimum length of bases required in the clear range of a sample file. Any less than this number causes the sample to fail analysis.
Minimum Sample Score	Minimum quality value score (average of all sample QVs in the clear range) that is acceptable. The range is 1–50 (see “Sample Quality Values” on page 10-3

3. When the analysis protocol is complete, click **OK** to save the new settings. If you do not want to save the new settings, click **Cancel** to save the previous settings.

   Note: To implement the changes, you must click OK to save them and then run the analysis.

   If all filters pass, then the assembly occurs.

Specifying the Analysis Settings

To accommodate sample variability and to ensure the quality of your results, you can modify the settings used to analyze a sample and then reapply them to a sample.

You can save changes to the analysis defaults and display settings contained in a project, and you can also save them in SeqScape Manager to be used in a project template.

The procedures in the following sections describe selecting the analysis settings for a set of samples. These analysis settings can be saved as analysis defaults and saved in SeqScape Manager.

For information on reapplying a new project template, see "Reanalyzing a Project Using a Different Project Template" on page 6-24.

Gap and Extension Penalties

Project Tab Settings

The settings for Gap Penalty apply for alignment of different specimen consensus sequences to each other and the reference.

If you want to add gap and extension penalties, these settings introduce gaps into sequence alignments allowing the alignment to be extended into regions where one sequence may have lost or gained characters not in the other gap penalty score (G+Ln). G is gap penalty, L is the length of gap, and n is the number of bases. A penalty is subtracted for each gap introduced into an alignment because gap increases uncertainty into an alignment.

Note: The default settings are already optimized for the current algorithm.

Specimen Tab Settings

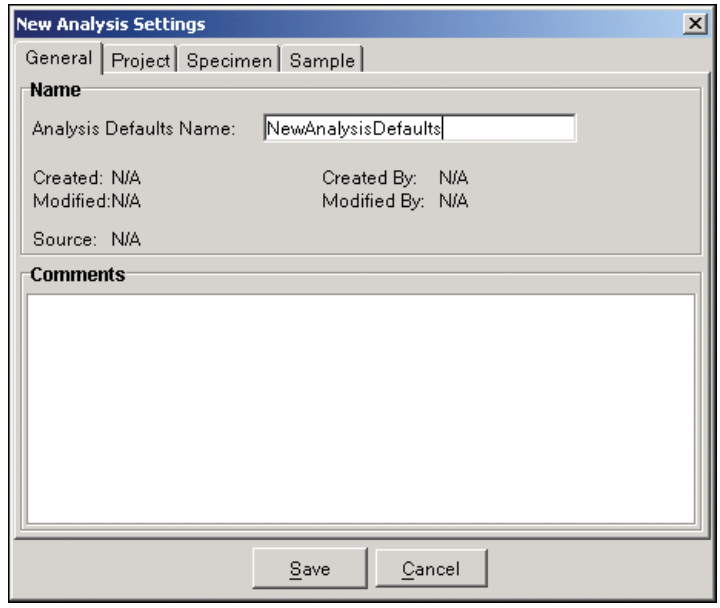
The settings of gap and extension penalty apply to setting alignment of samples to the reference.

Setting Analysis Defaults

To create new Analysis Defaults:

1. In the SeqScape Manager, select the **Analysis Defaults** tab, then click **New**.
2. In the General tab of the New Analysis Settings dialog box, enter an Analysis Defaults Name.

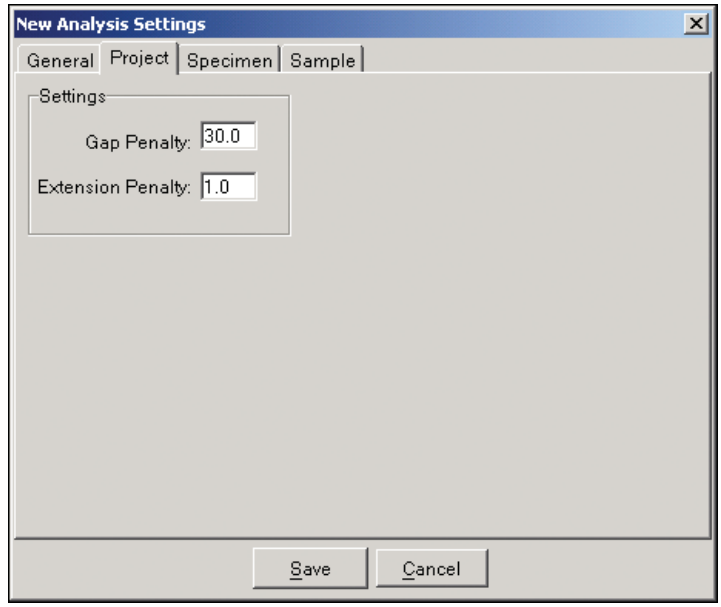
   Note: The name cannot contain spaces or characters that do not conform with the Windows file system. Refer to "File-Naming Convention" on page 2-9.



3. Enter any comments pertaining to the new analysis settings in the **Comments** box.

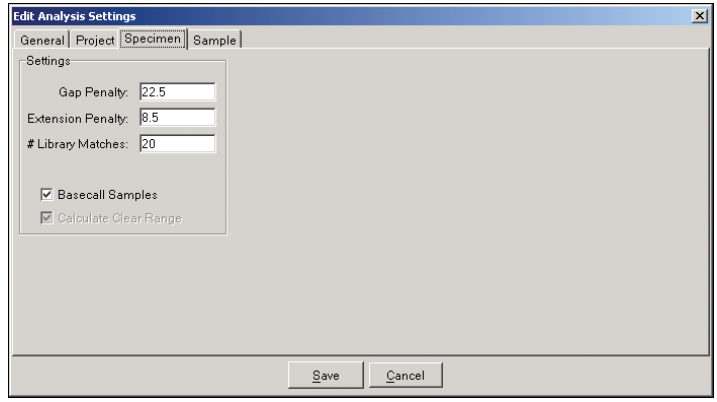
4. Select the **Project** tab and, if desired, change the Penalty Settings.

   Note: The gap and extension penalties refer only to the alignment algorithms that are used to align the consensus sequences to the references and to each other. They do not affect the alignment of the samples to the reference for assembly.



5. Select the **Specimen** tab.

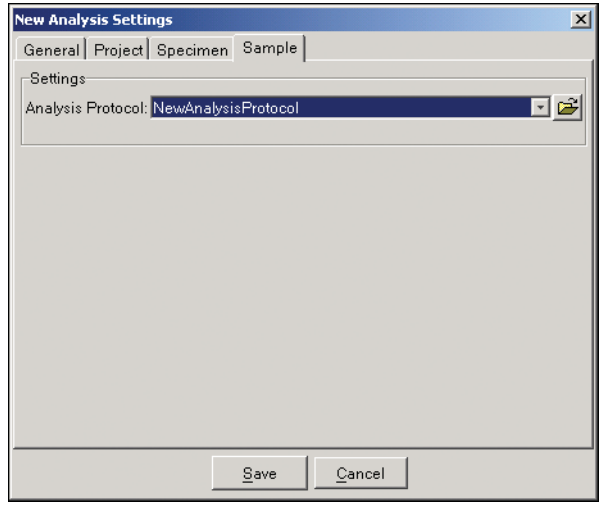
   The # Library Matches check box indicates the number of hits desired to match the library you select.



   a. If desired, change the settings.

   b. Select **Basecall Samples** to automatically calculate clear range and basecall samples. If you do not select Basecall Samples, the sample files are not basecalled, and it is assumed that you have previously basecalled and edited the data. When basecalling is skipped, the software proceeds to filtering and assembly in the analysis pipeline.

6. Select the **Sample** tab then select the analysis protocol you just created from the Analysis Protocol drop-down list.



7. Click **Save** to save the new settings for this project.
8. Click **Close** in the SeqScape Manager dialog box.

Selecting the Analysis Default Settings for Individual Samples

Note: Changing the analysis defaults does not affect the analysis settings of samples that are already in the project.

To select the analysis settings for each sample individually:

1. Select the sample in the Project view.
2. Select **Analysis** > **Analysis Settings**. This opens the Analysis Protocol for that individual sample file.
3. Make relevant changes to the settings, then click **Save**.

Specifying Display Settings

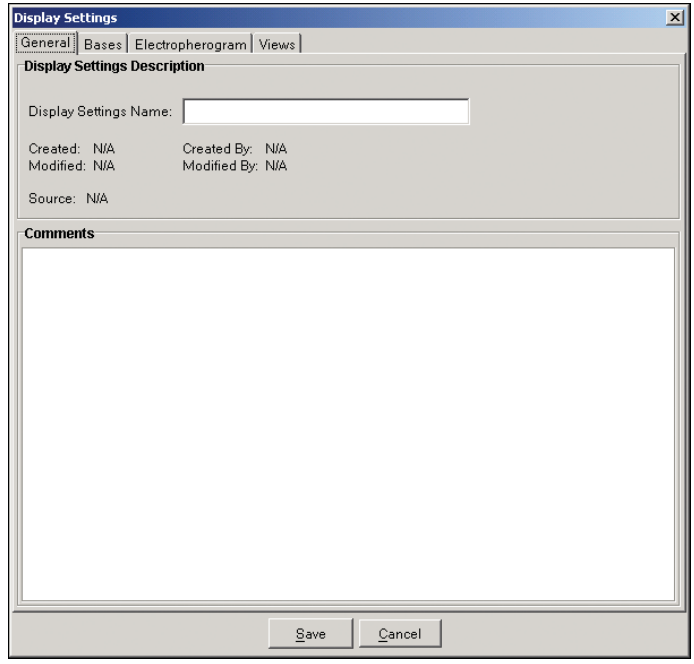
To accommodate personal preferences, the SeqScape software allows you to select the way results are displayed. The display settings can be modified and then reapplied to a project. The selected settings can also be saved in the SeqScape Manager to be used in a project template.

The display settings control:

- Font colors and style for bases
- Electropherogram display and axis scale
- Display views for variants
- Display views for nucleotide translation
- Quality value display and thresholds

To specify the display settings:

1. Select **Analysis > Display Settings** in the main SeqScape window. The Display Settings dialog box opens displaying the General tab.

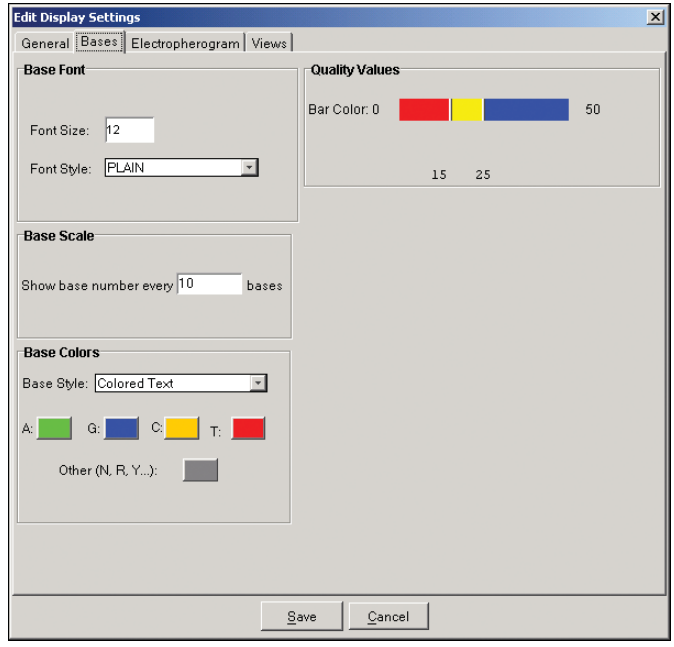


2. Click the **Display Settings Name** field, then enter a name for the new display settings.

   IMPORTANT! The name cannot contain spaces or characters that do not conform with the Windows file system. Refer to "File-Naming Convention" on page 2-9.

3. Enter any comments you want to record for the sample.

4. Select the **Bases** tab.



a. Enter the desired text style and color for each base. In the Base Font section, select your font preferences for the sequence bases, or use the defaults.

b. In the Base Scale section, enter the frequency at which to display bases for the reference sequence in the Project view.

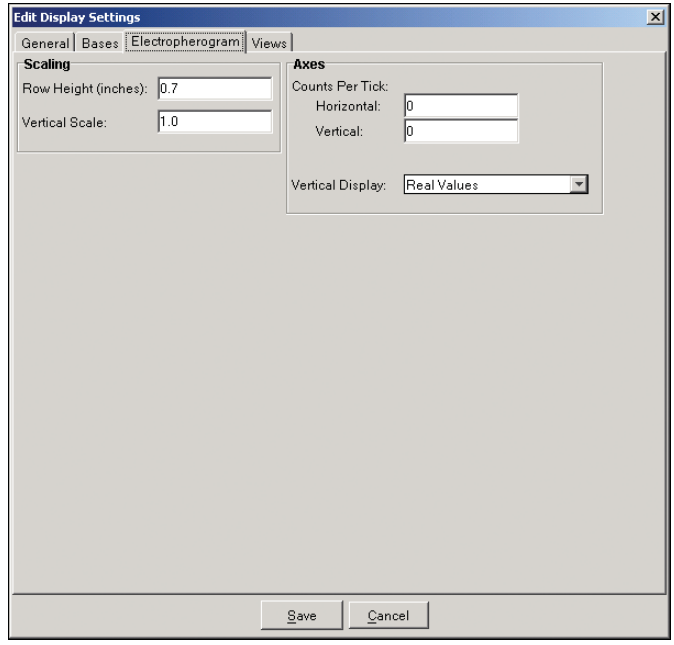
c. In the Base Colors section, select your color preferences for the sequence bases and electropherogram traces. To select a color, click the colored box (next to A:, G:, C:, and T:) to open the color chart. Select a new color, then click **OK**.

d. In the Quality Values section, click the colored bars to open a color chart, then select the color, if necessary. To select the threshold values, drag the divider bars between the colors.

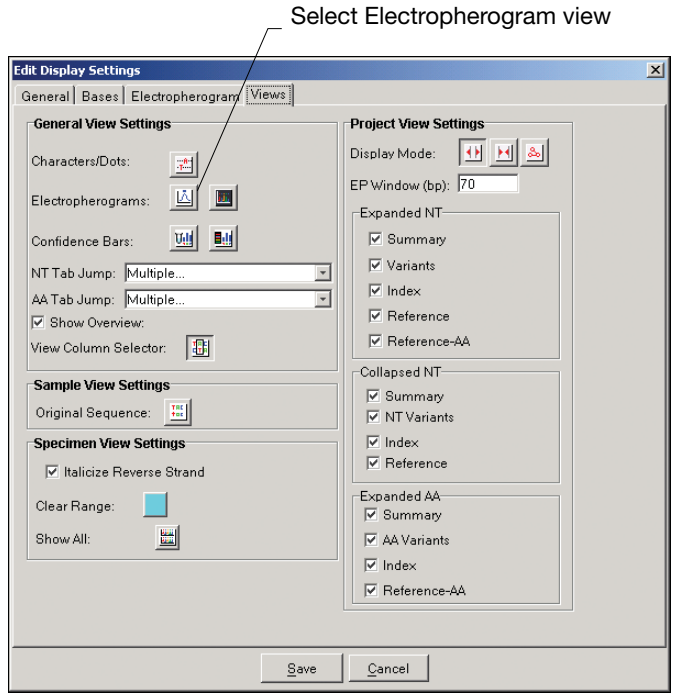
Note: The styles you specify here do not apply to variants.

For more information on quality values, see Chapter 10, “Sample and Consensus Quality Values.”

5. Select the **Electropherogram** tab, then enter your preferences for viewing the electropherogram and axes.
   a. Enter your Scaling and Axes preferences.
   b. Select a Vertical Display setting (**Real Values** or **Relative**).



6. Select the **Views** tab, then enter your preferences for the Project and Specimen views.



a. In the General View Settings section, click the buttons for the displays you want turned on in the project. Select the **Electropherogram** view.

   Note: If it is not selected, when the Assembly view is printed, it appears that the peaks are not aligned.

   Most of the buttons on this tab are the same as the viewing buttons on the lower row of the toolbar in the main SeqScape window. Refer to “Viewing Toolbar” on page 2-23

b. In the drop-down menus, select how you want to tab through the data.

c. In the Sample View Settings section, select the icon if you want to see the original sequence displayed.

d. To differentiate forward and reverse sequences, in the Specimen View Settings section, select **Italicize Reverse Strand**.

e. In the Project View Settings section, enter the number of bases to be displayed for the Project view electropherogram snippets in the EP Window field (the minimum is 3).

7. Click **OK** to save the changes to the open project and close the dialog box.

IMPORTANT! To save your modified settings as a new set of display settings in the SeqScape Manager, click **Save To Manager As**, then name the set.

Creating a Reference Data Group

4

This chapter contains:

Workflow for This Chapter

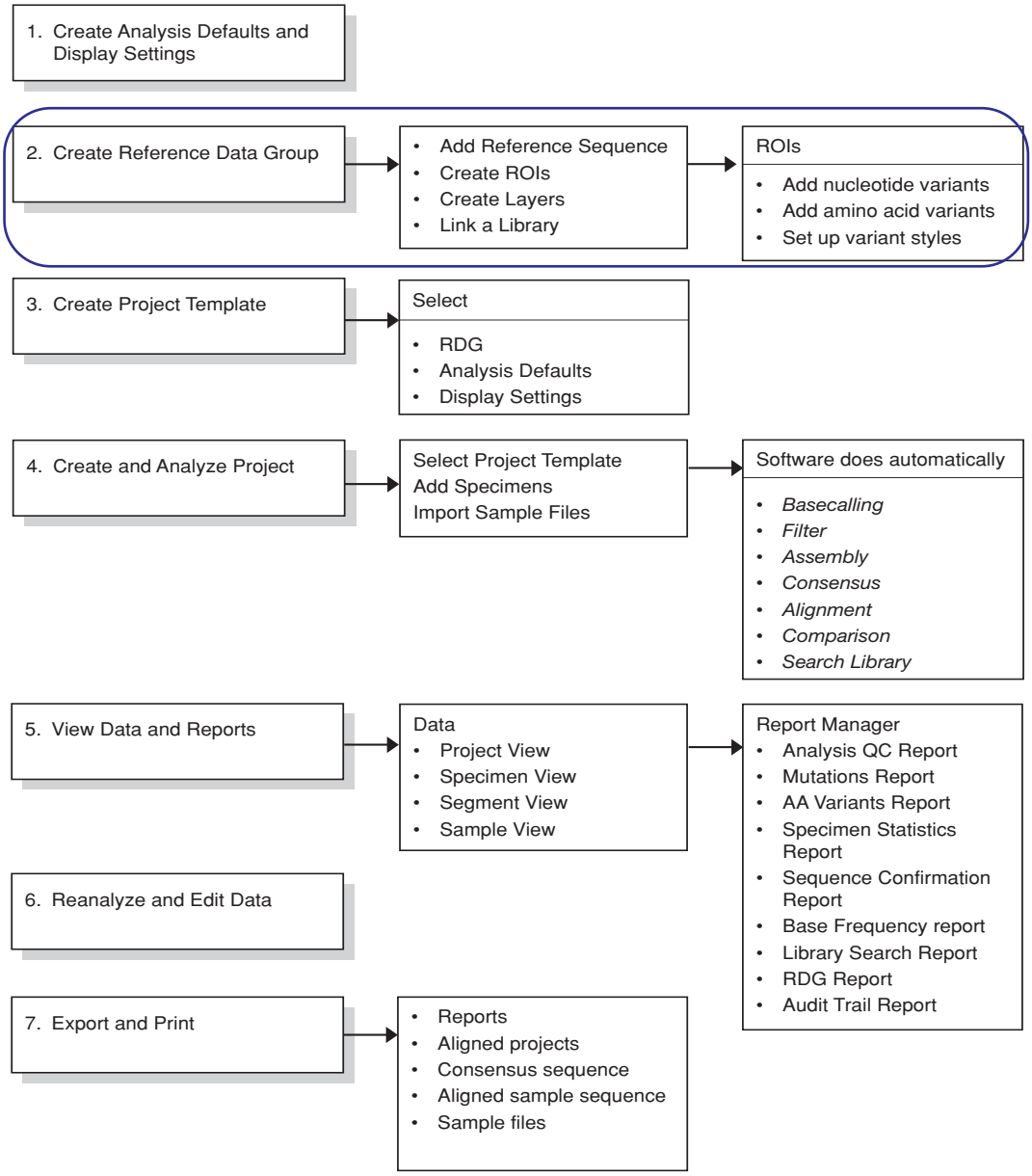


Figure 4-1 Showing Create Reference Data Group Step

Reference Data Group (RDG)

About the Reference Data Group

The Reference Data Group defines the sequence to which the ABI PRISM® SeqScape® Software Version 2.0 compares the consensus segments to the reference sequence. It contains the reference sequence and reference-associated data. The reference sequence is the entire "backbone" sequence for the project, consisting of one or more reference segments separated by reference breaks.

The RDG contains all the gene/analysis-specific information consisting of:

- A reference sequence containing continuous or discontinuous sequences made up of one or more reference segments
- Nucleotide variants
- Amino acid variants
- Translation codon table
- Layers, which are units of analysis in any project, and regions of interest (ROIs) grouped together into layers for display and translation
- Associated allele libraries
- User-defined styles for identification of variants in the project

A reference segment is a contiguous segment of the reference sequence corresponding to a single contiguous DNA sequence. It is also a region of interest. The reference segment consists of:

- An analyzed sample file
- A text-only format, FASTA, or .seq file
- A GenBank format file

GenBank Features

Every GenBank entry has a single contiguous sequence associated with it. This is also referred to as the source feature. This sequence is always numbered starting at 1.

Because of this, the sequence from a single GenBank entry translates into a single reference segment in the extended RDG. Numbering of the base ROI on this segment is set by default to start at 1.

Every GenBank entry has a feature table. These features translate into regions of interest and layers in the extended RDG. In the following table, items in {} are qualifiers read for that feature key (for example, {gene} is the value of the `\gene` qualifier). If that qualifier doesn't exist, then "" is substituted.

Table 4-1 GenBank feature table

GenBank Feature	Extended RDG equivalent
source	Skipped. The source feature corresponds to the region of interest associated with the whole reference segment that is automatically created.
exon	Region of interest is created, called {gene}_exon{number}. Translatable by default.
intron	Region of interest is created, called {gene}_intron{number}. Not translatable by default
gene	Region of interest is created, called {gene}_gene. Translatable by default.
CDS	Layer is created, called ({gene}|{product})_CDS. If translatable regions of interest exist that correspond to this CDS, then those are used for building the layer. Otherwise, new regions of interest are created as required. New ROIs are called {layerName}_region1, {layerName}_region2, etc… Translation frame and orientation is taken from CDS qualifiers (`complement`() and `\codon_start`).
misc_feature	Region of interest is created called {note}. Not translatable by default.
Unknown feature	Region of interest is created called {feature key}. Not translatable by default.

It is possible with this translation table to create many non-uniquely named ROIs (for example, if the entry had lots of variation features).

Downloading a GenBank File

To download a GenBank file from the Internet:

1. Open your web browser and enter the following URL:

   `http://www.ncbi.nlm.nih.gov/`

2. In the Search menu, select **Nucleotide**.
3. Click the **for** text box, then enter the nucleotide you want for the reference sequence.
4. Select the GenBank file check box for the file you want, then click **Save**.
5. Make sure **Save this file to disk** is selected, then click **OK**.
6. Name the file using the .gb extension, then navigate to the directory on your computer to save the file.
7. Click **Save**.
8. When the download is complete, click **Close**.
9. Download additional files or quit your web browser.

About Creating a New Reference Data Group (RDG)s

IMPORTANT! Only a user from the Administrator or Scientist group can set up a new RDG. Refer to Appendix D, "User Privileges," for a list of the privileges that apply to each group.

You can create a new RDG in the following ways:

- Use the RDG wizard and follow the instructions
- Use the SeqScape Manager window to open a blank RDG

Follow the RDG wizard procedures below, if desired, to familiarize yourself with the windows of the RDG. Then, create subsequent RDGs by using the SeqScape Manager. Refer to "Creating a New RDG Using SeqScape Manager" on page 4-12.

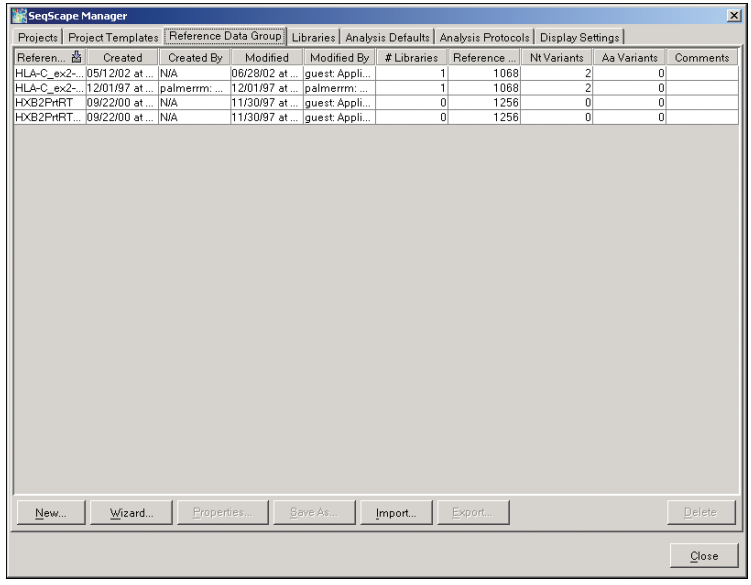
Creating a New RDG Using the Wizard

Using the Wizard to Learn the Software

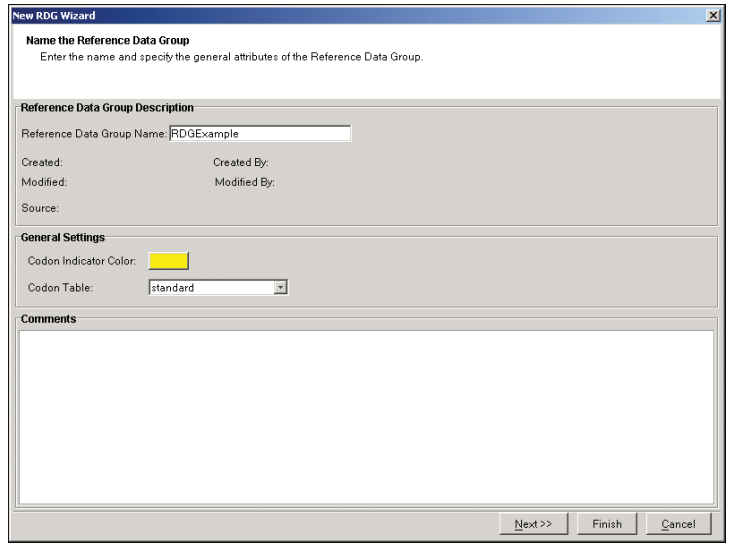
The wizard in the SeqScape Manager will familiarize you with setting up a new RDG.

To create an RDG using the RDG wizard:

1. In the main SeqScape window, select **Tools > SeqScape Manager**.
2. Select the **Reference Data Group** tab, then click **Wizard** at the bottom of the page.



3. Enter a name for the new RDG that conforms with the Windows file system. Refer to “File-Naming Convention” on page 2-9.

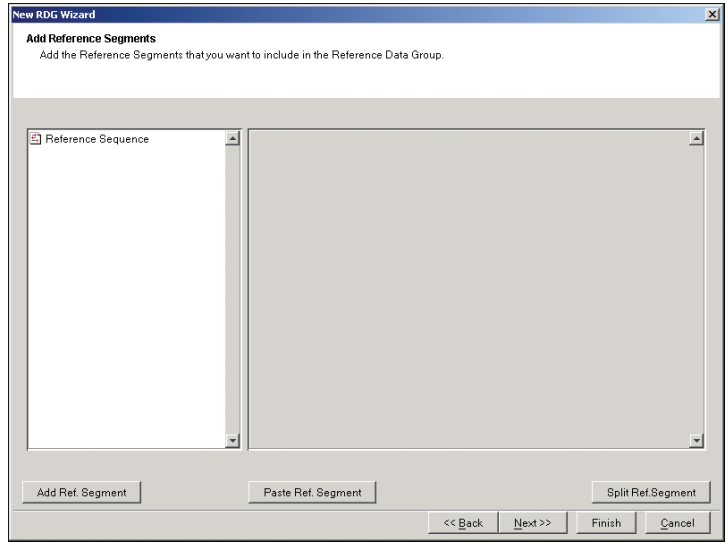


4. If desired, click the Codon Indicator Color by clicking the yellow color box, and select a new color.

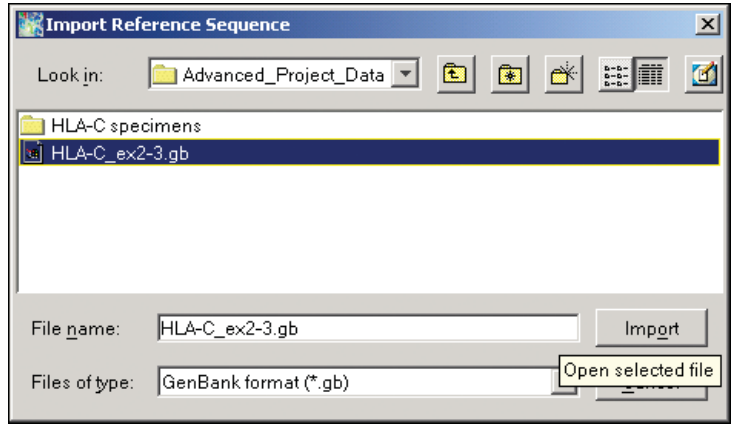
5. Then select the Codon Table to use.

6. Click **Next**. The next page shows the Reference Sequence pane. The Reference Sequence forms the backbone for comparison. It is made up of one or more reference segments.

7. Click **Add Ref. Segment** in the lower left to add a segment to the Reference Sequence. A reference segment is a single sequence imported from a text file or GenBank file.

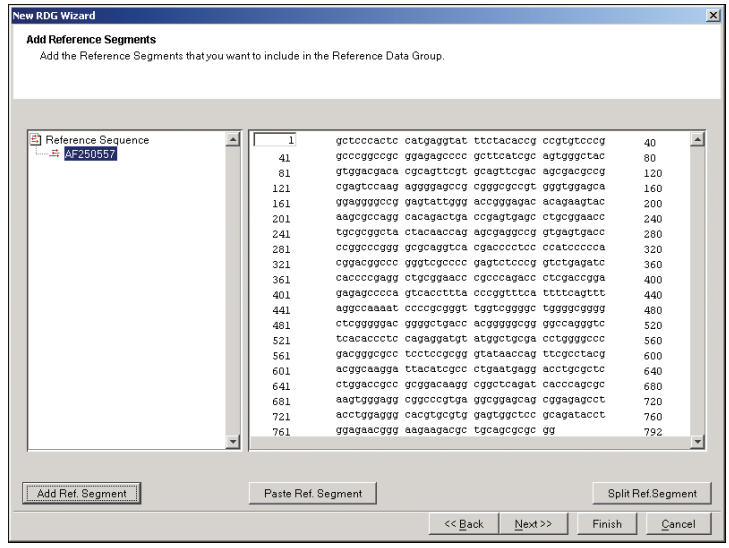


8. Navigate to the file containing the reference sequence that you have stored, such as a GenBank file (the file may have a .gb extension).



IMPORTANT! The window opens to the directory that was set up during installation of the software. If no default directory has been specified, the window opens to the C:\ drive. If you need to set up the default directory, select **Tools** > **Options** > **General**, then click **Browse** to locate the directory.

9. Click **Import**. The imported sequence appears in the right pane of the dialog box, as shown in the figure below.



10. Click **Next**.

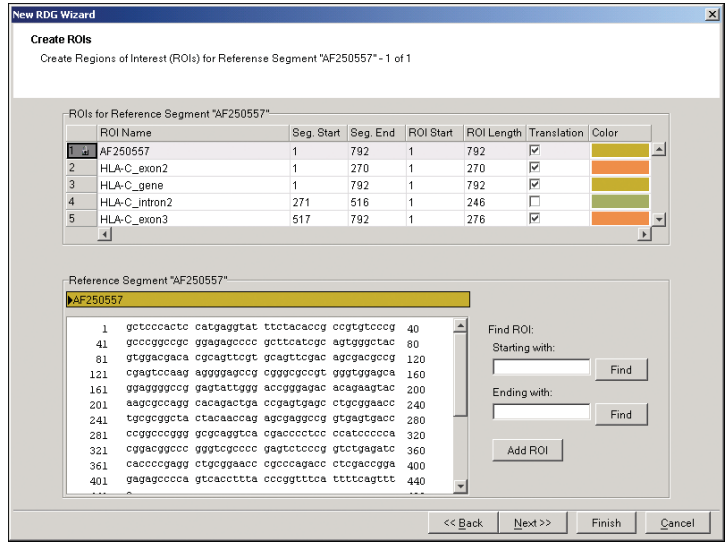
Note: For a procedure on using the Paste Ref. Segment button, refer to “Pasting a Reference Segment” on page 4-15, and for a procedure on using the Split Ref. Segment button, refer to “Adding a Reference Break in a Sequence” on page 4-27.

The wizard continues the instructions to add a new layer and regions of interest (ROI) to that layer. An ROI is a region on a reference segment that defines exons, introns, splice junctions, and other features.

Setting Up the Reference Segment

To set up the reference segment:

1. Select the bases in the region of interest that you want to compare with the reference sequence (or backbone). In the Reference Segment pane, drag through the bases you want to select, or type the starting and ending bases under the Find ROI label.

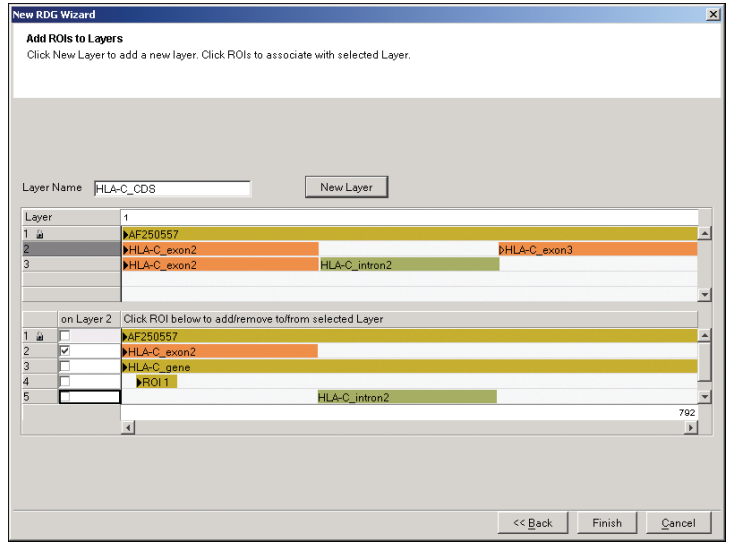


2. Click **Add ROI** to add the segment to the ROI table in the ROI pane above the sequence. Add as many ROIs as desired.
3. Click **Next**.
4. Follow the instructions to add layers and ROIs to layers. Layer 1 is always the reference sequence, which is generated by the software and is locked. Click **New Layer**, then name each layer that you add.

   IMPORTANT! To avoid confusion, give each layer that you add a unique name.

5. Click the new layer under the Layer label in the layer pane, then select the ROI **on Layer** check box in the ROI pane to associate it with the selected layer. Do this for each layer you create.

   IMPORTANT! In a layer, you cannot define ROIs that overlap one another.



6. Click **Finish**, or if you want to change any of the selections, click **Back**.

   The newly created RDG appears in the Reference Data Group list.

Creating a New RDG Using SeqScape Manager

Before You Begin

You must have administrator or scientist privileges to create a new RDG using SeqScape Manager.

Before creating a new RDG, make sure you:

- Download a GenBank file, a FASTA text file, or have a reference sequence that is stored on your computer
- Define on paper the ROIs, layers, and segments to compare to the reference sequence

Creating an RDG from SeqScape Manager

Creating a Reference Data Group, requires that you:

- Import reference segments
- Create ROIs
- Create layers

To create a new RDG from the SeqScape Manager:

1. In the main SeqScape window, select **Tools** > **SeqScape Manager**, then select the **Reference Data Group** tab.
2. Click **New**.
3. In the General tab, enter a name in the Reference Data Group Name field. The General tab contains general information about the RDG.
4. Select a Codon table type and add comments, if desired.
5. Select the **ROI** tab.

IMPORTANT! Do not click OK. More steps are needed to set up the RDG.

About the Reference Sequence

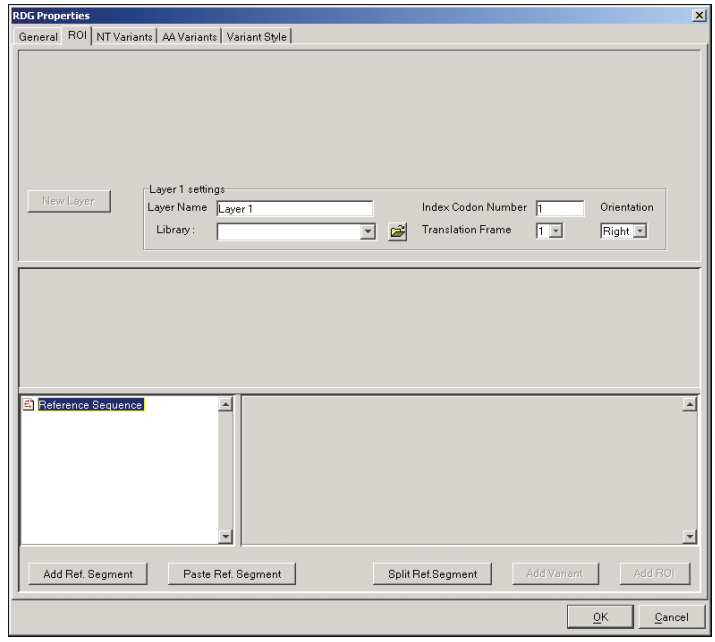
The reference sequence is made up of one or more reference segments that become a backbone or reference to which all other sequences or regions of interest are compared. After the reference sequence is imported into the RDG, it cannot be changed or edited.

Importing a Reference Segment

To form the reference sequence, you need to import one or more segments.

To import a reference segment:

1. If it is not already open, select the **ROI** tab. The dialog box that opens shows Reference Sequence as a place holder in the lower left pane.



2. Click **Add Ref. Segment** in the lower left to add a segment to the reference sequence.
3. Navigate to the file containing the reference sequence. It can be a GenBank file or a file that you stored on your computer (the file may have a .gb extension).
4. Click **Import**.

   The reference sequence is on Layer 1, which is locked so it cannot be modified.

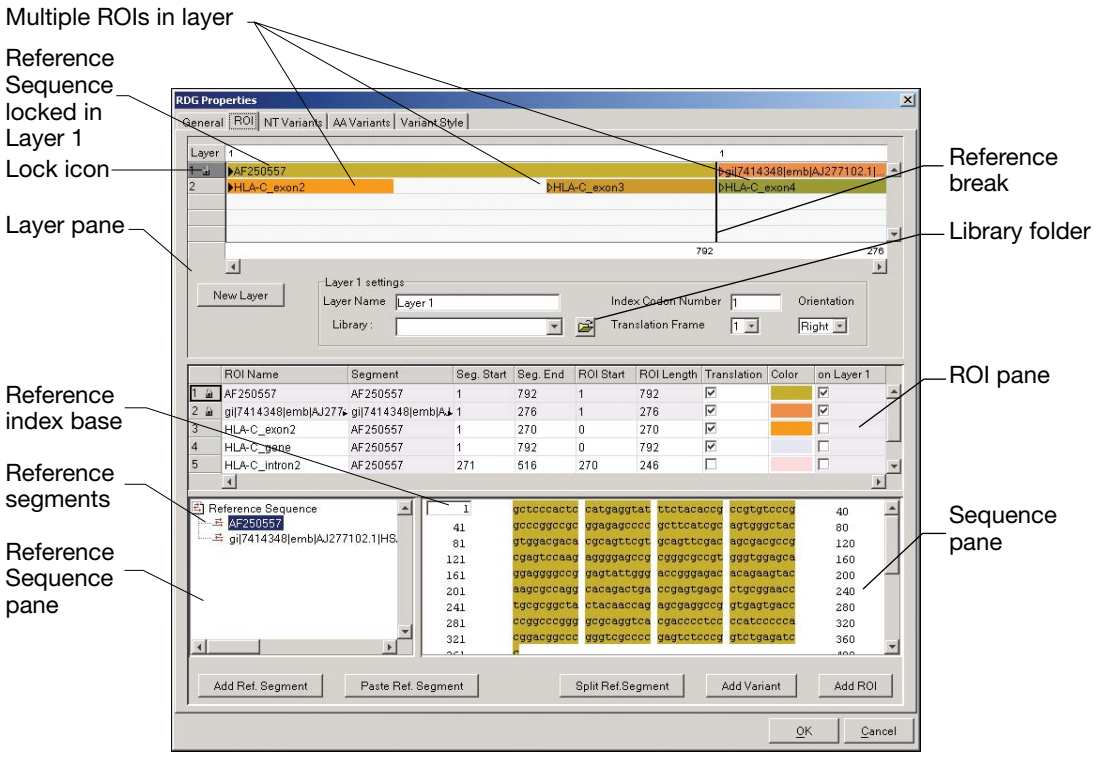


Figure 4-2 ROI Tab in the RDG Properties Dialog Box

Defining Regions of Interest (ROI)

Defining an ROI

Each reference segment has its own locked ROI. Identify the ROIs you want to define on a piece of paper, then use the information to define ROIs in the software. However, if you are using a GenBank file, the ROIs or features will already be defined. You can add additional ROIs where appropriate to your analysis.

To define an ROI:

1. In the ROI tab, select an empty layer or a layer where you want the ROI to appear.
2. Select a segment in the nucleotide sequence pane (by dragging through the region of interest), then click **Add ROI**.
3. Enter a name for the ROI under the ROI Name column in the ROI pane.
4. Define as many ROIs as appropriate by dragging through the regions of interest, or by entering a number in the text box where the ROI should begin.

Pasting a Reference Segment

You can create or enter a sequence in a text editor or word processing program and copy the segment into the RDG at a later time.

To define a reference segment by pasting:

1. Open a text file, then click-drag the region of interest you want to use as a reference segment.
2. Select **Edit** > **Copy**.
3. In the RDG Properties ROI tab, click **Paste Ref. Segment** to use a reference segment that you copied to the clipboard. The copied reference appears in the Reference Sequence pane.
4. If you want to delete the copied reference segment, select it, press **Delete**, then click **OK** in the Confirmation dialog box.

Deleting an ROI or Layer

To delete an ROI or layer, reference segment:

1. Select the ROI, layer, or segment.
2. Press **Delete**. Only unlocked rows can be deleted.
3. Check this on software

   IMPORTANT! After you delete an object, it cannot be undone.

Deleting a Reference Segment

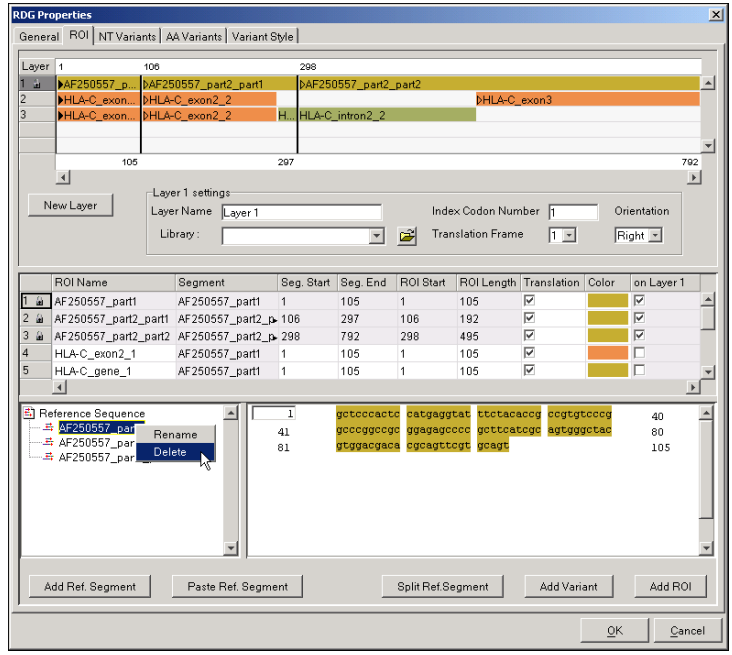
When RDG Properties window is open without being associated with a project, a reference segment can be deleted. Right-clicking a selected reference segment in the Reference Sequence pane opens a pop-up menu with selections to rename or delete the selected segment.

Note: The reference segments and Layer 1 cannot be deleted by selecting them in the Layer pane, then pressing Delete, because they are in locked layers. They cannot be deleted when they are part of an existing open project.

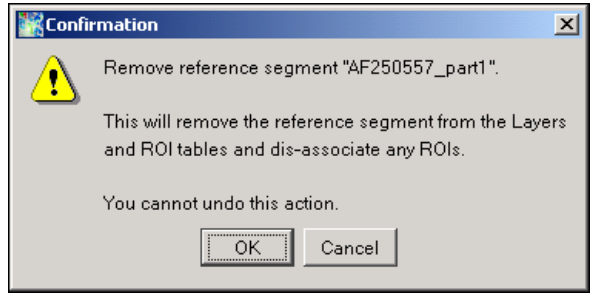
To delete a reference segment:

1. In SeqScape Manager, select the **Reference Data Group** tab and highlight the RDG in which you want to delete a reference segment.
2. Click **Properties**, then click the **ROI** tab.
3. In the Reference Sequence pane, highlight the reference segment you want to delete.

4. Right-click and select **Delete** in the pop-up menu.



A confirmation dialog box opens.



5. Click **OK** in the confirmation dialog box. Once you click OK, the delete cannot be undone.

ROI Tab Descriptions

Layer Pane Functions

The Layer pane in the ROI tab (refer to Figure 4-2 on page 4-14) has the following functions:

- Layers – Shows the locked Reference Sequence in Layer 1, and ROIs associated with each layer.
- New Layer button – Adds a new layer to the end of the layer table.
- Layer Number Settings – The settings of the selected layer. Each layer has its own unique settings.
- Layer Name – The name of the layer, which can be edited.
- Library – Contains libraries to select if you are performing allele or haplotype identification. Before you select a library to associate, the Library field is blank. A library can be copied into the RDG, but is not associated until you select it from the Library drop-down list. Once it is selected, the Library field shows the name of the library.
- Index Codon Number – Indicates the first amino acid number. This number is always in relation to the number of the first reference segment base, positive numbers only.
- Translation Frame – Sets the translation frame for the layer. The values are 1, 2, 3.
- Orientation – Sets the orientation of the layer, right (forward) or left (reverse).

The ROI Pane

The ROI pane has the following features:

- Clicking a row selects the ROI. When you select an ROI in the RDG, it selects and scrolls the reference segment and the associated sequence.
- Primary ROIs that are created when reference segments are imported are locked as indicated by the lock icon. These primary ROIs cannot be deleted from the ROI table, but can be deleted from the Reference Sequence navigation pane by right-clicking and selecting Delete.

Columns in the ROI Pane

The ROI pane in the middle of the RDG Properties dialog box has the following columns:

- ROI numbers – The number of the ROI. The Reference Sequence on Layer 1 is always locked. Reference segments that make up the Reference Sequence are also locked. Unlocked layers are below the reference segments and can be edited.
- ROI Name – Name of the ROI. ROI names that are not locked can be edited. The ROI Name must be unique.

  Note: Names for Reference Segments are not editable in the ROI pane. They can be edited in the Reference Sequence navigation pane by right-clicking and selecting **Rename**.

- Segment – Name of the segment to which the ROI is associated.
- Seg. Start – The nucleotide number in the Reference Sequence where the ROI begins.
- Seg. End – End of the ROI segment.
- ROI Start – The first nucleotide number you assign to this ROI. The number can be positive or negative.
- ROI Length – Length of the ROI. The value is automatically recalculated if you change the Segment Start or ROI Length values. Entering a number into this cell automatically recalculates the ROI Length value.
- Translation – Sets whether or not the ROI is translated.
- Color – Shows the color of the ROI. Click to display the standard Color Picker dialog box if you want to select a different color for the ROI.

  Note: When an ROI is defined, a default color is applied to the ROI based on the name of the ROI.

- On Layer (number) – Check box. The label for this column changes based on the selected layer. If the check box is selected, the ROI appears on the selected layer. ROIs can be associated with multiple layers. However, ROIs cannot overlap on a layer. Therefore, the check box is disabled if the Start/Length range of the ROI overlaps with the range of an ROI already associated with the layer. This prevents you from overlapping ROIs on the Layer table. A dialog box appears if you try to select an overlapping ROI.

Creating a Library

About the Library

You must classify your library as a haploid or diploid library and determine how many library matches you would like to see for each consensus sequence. A library match is one allele or a pair of alleles that agree closely with each consensus sequence.

A haploid library contains sequences that have pure bases only (AGCT). When searching against a haploid library, SeqScape software provides library matches, and each library match contains a pair of sequences (haplotypes) that best match the genotype of each consensus sequence.

A diploid library contains sequences composed of pure bases only, or pure bases and mixed bases. When searching against a diploid library, SeqScape software provides library matches, and each match is a single sequence that best matches the genotype of each consensus sequence.

Using Aligned FASTA Files

To use the library search feature, you must import an aligned multiple sequence FASTA file into the SeqScape software. If you have a series of FASTA sequences, you must use a tool to align those sequences and create a single aligned multiple sequence FASTA file before importing the file into the software.

Using a Tool to Align the Files

If you have a series of text sequences or electropherograms, you must create FASTA files, then use a tool to align those sequences and create a single multi-aligned FASTA file.

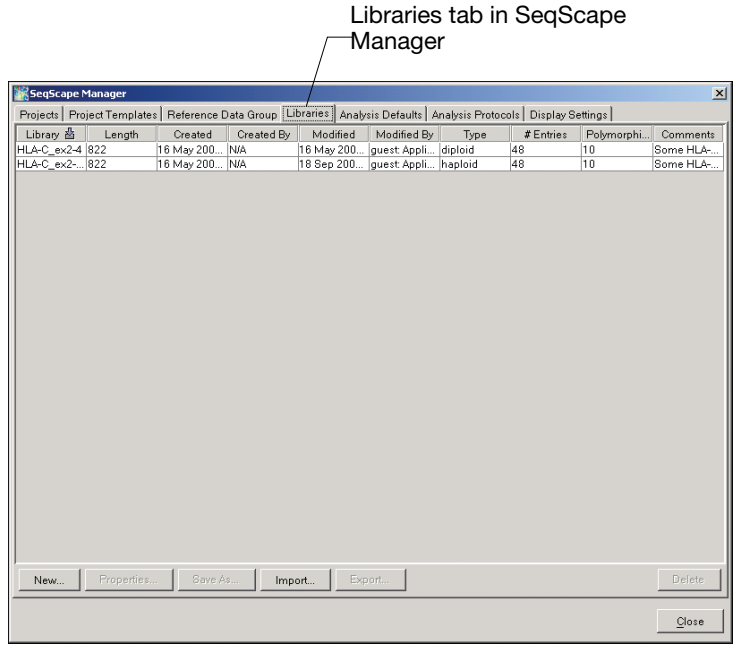
A common tool used to create aligned multiple-sequence FASTA files is Clustal X. Given individual FASTA files, the application generates an aligned multiple sequence FASTA file with all sequences in equal length. The tool was developed by the National Center for Biotechnology Information as part of their NCBI Software Development Toolkit. The toolkit is available by anonymous ftp from ncbi.nlm.nih.gov. You can locate this free tool by using your Internet browser and search for “Clustal X”.

Setting Up Your Library

Note: Review the following procedure, "Setting Up Your Library," then go to the next section, "Creating New Layers" on page 4-24. Use the following procedure to select the library before continuing with the procedure to create new layers.

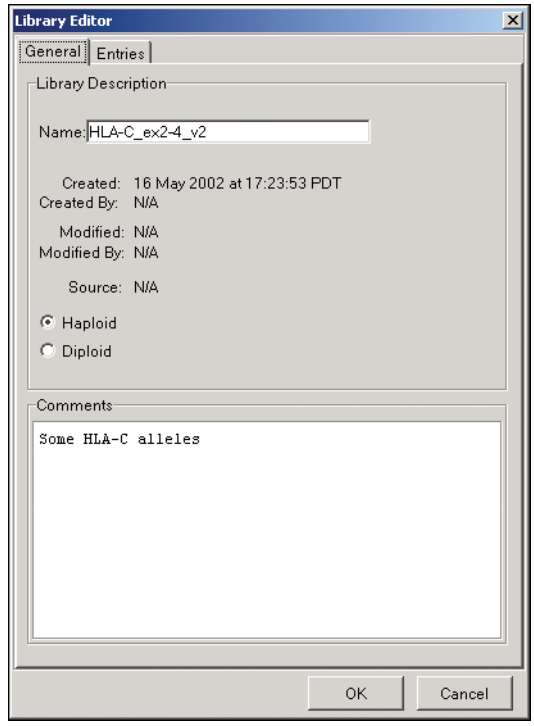
To set up a library:

1. In the main SeqScape window, select **Tools** > **SeqScape Manager**, then select the **Libraries** tab.



2. Select **New**.

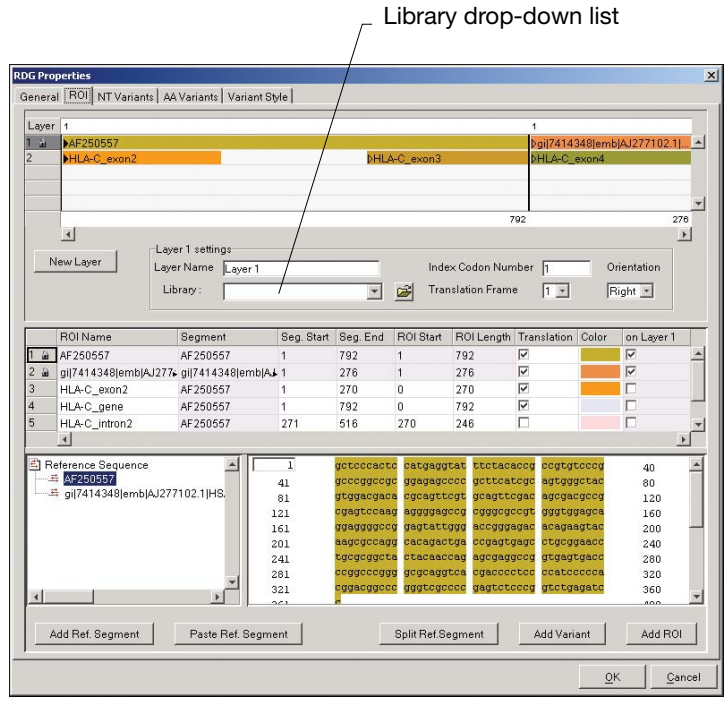
3. In the Library Editor General tab, enter a name for the new library, and select **Haploid** or **Diploid**.



4. Select the **Entries** tab, then click **Import**.
5. Import the aligned multiple sequence FASTA file, and click **OK**.
6. In SeqScape Manager, select the **Reference Data Group** tab and select the RDG that you want to link to the library
7. Click **Properties**, then select the **ROI** tab.

   Note: At this point, if you do not have layers in the RDG or you do not know how to create a layer, go to "Creating New Layers" on page 4-24. Otherwise, continue to the next step.

8. Select a layer in the Layer pane.
9. In the Library drop-down list, select the corresponding library that you created in steps 2 through 5.

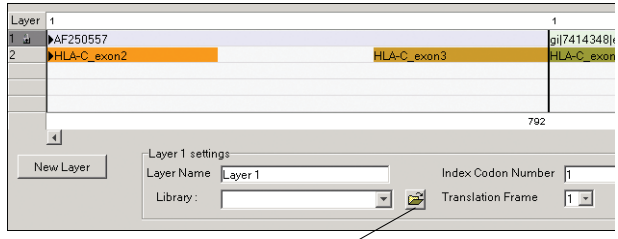


Creating New Layers

Layers organize groups of related, nonoverlapping ROIs. By organizing ROIs into layers, results reviewing and library searching are faster and more focused. The Layer table shows the organization of ROIs into layers.

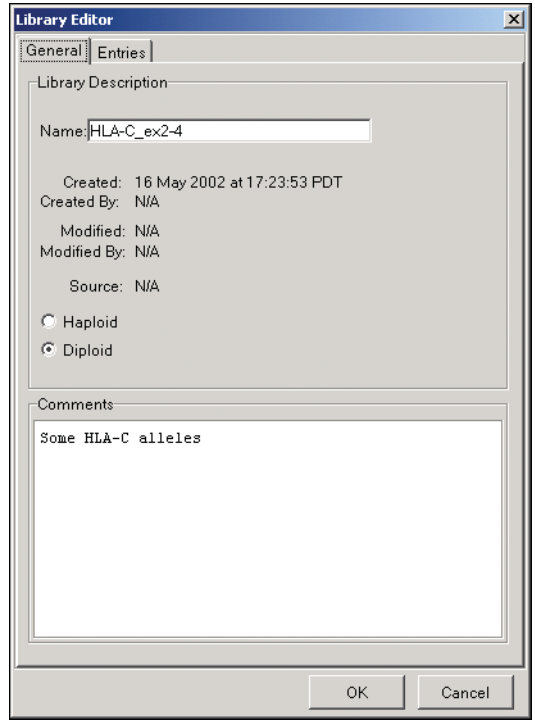
To create new layers:

1. In the ROI tab, Layer pane, click **New Layer**, then enter a name in the Layer Name field.
2. Select a layer by clicking it under the Layer label in the Layer pane. If you need more information on libraries, refer to "Creating a Library" on page 4-20.
   a. Select a library from the Library drop-down list if you are performing allele or haplotype identification.
   b. Select the library folder icon to open the Library Editor and view the entries.



Note: The selected library in the Library drop-down list is associated with the layer in the Layer Name field.

The Library Editor opens as shown in the sample below.



c. In the Layer pane, enter the Index Codon Number.

d. Select the Translation Frame.

e. Select the Orientation.

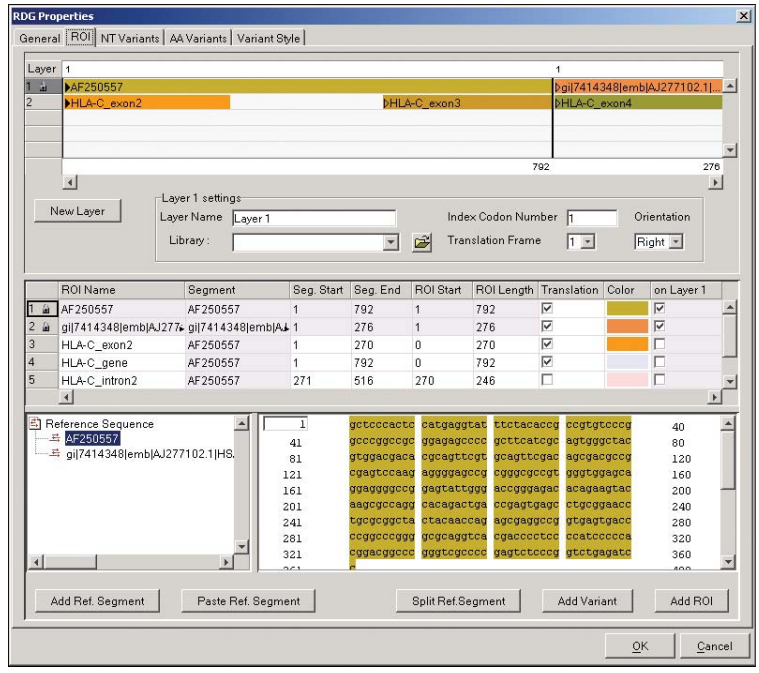
3. Select the appropriate Reference Segment in the Reference Sequence pane, highlight the sequence representing the ROI, then click **Add ROI**.

   The ROI is added to the ROI pane and to the selected layer.

4. Repeat the process to build layers containing all the ROIs and layers you previously defined on paper.

5. You can edit the ROIs in the ROI pane by selecting the attributes, then editing them directly in the table.

6. To include an existing ROI on an unlocked layer, select or create the layer, then select the **On Layer** check box for the ROI.



IMPORTANT! If you want to add variants, follow the procedure on page 4-29. Do not click OK. If you do not want to add variants, go to the next step.

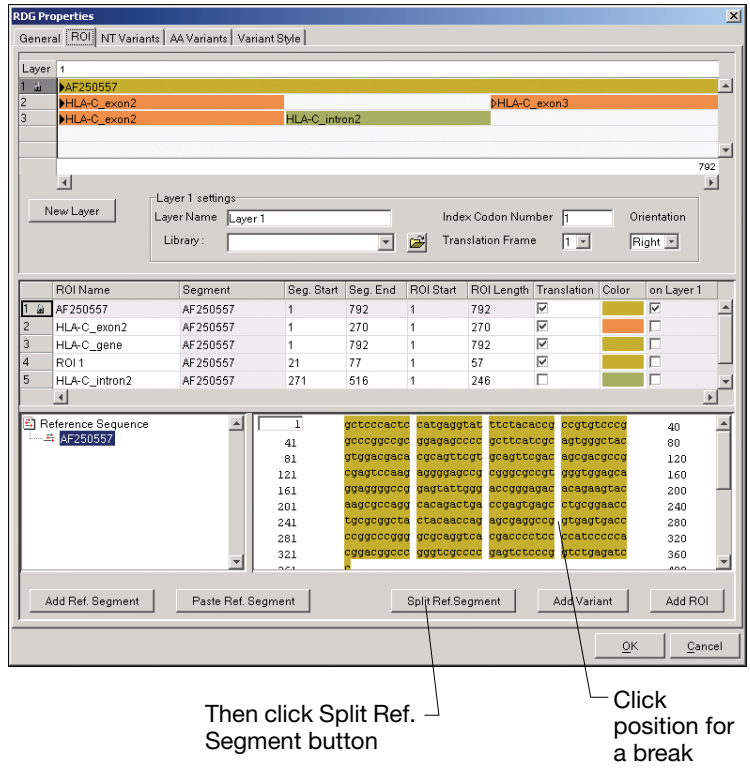
7. When you finish adding ROIs and layers, click **OK**. The new RDG appears in the Reference Data Group list.

Adding a Reference Break in a Sequence

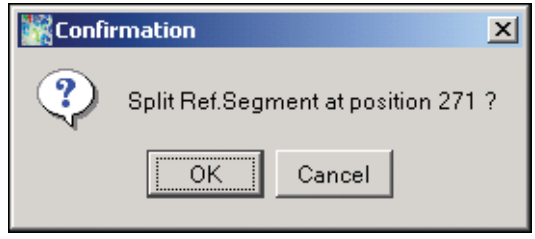
A reference break can be added in the Reference Sequence by using the Split Ref. Segment button. Reference segments can be split if you want to delete intervening reference sequences. When reference segments are split, the ROIs associated with the reference segment are also split.

To add a reference break:

1. In the ROI tab Sequence pane, select the base position where you want a split to occur, then click **Split Ref. Segment**.

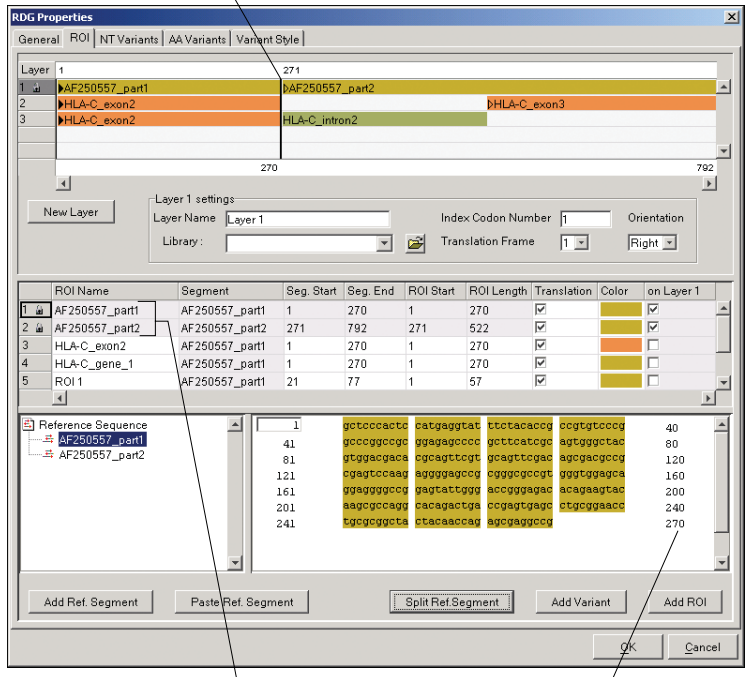


2. A confirmation dialog box opens showing the position for the reference break. Click **OK**.



There is a new reference break in the Reference Sequence in the Layer pane, as shown in the next screen shot. The Reference Sequence shown in the ROI Layer is now in two locked layers, one segment ending at position 270, and the second segment starting at position 271. The Sequence pane shows the first segment ending at position 270.

New reference break at position 271



Two segments in the Reference Sequence

First segment ending at position 270

Declaring Variants into an RDG

About NT Variants

The NT Variants tab in the RDG Properties dialog box lists the known nucleotide variants associated with a reference sequence. The entries you define in this tab are used to identify known and unknown variants in your projects.

You can enter NT variants by:

- Clicking Add Variant in the ROI tab, then entering the variant attributes in the New NT Variant dialog box.
- Creating a table of variants in a tab-delimited format, then saving the file and importing it into the NT variant file.

  One way of creating a table of variants is by using Microsoft® Excel. The columns in the Excel table must map to the columns in the NT Variants tab as shown below.

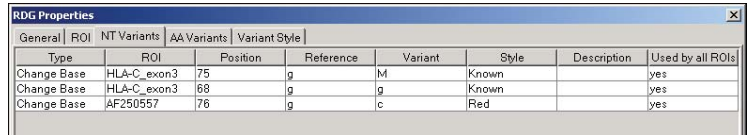


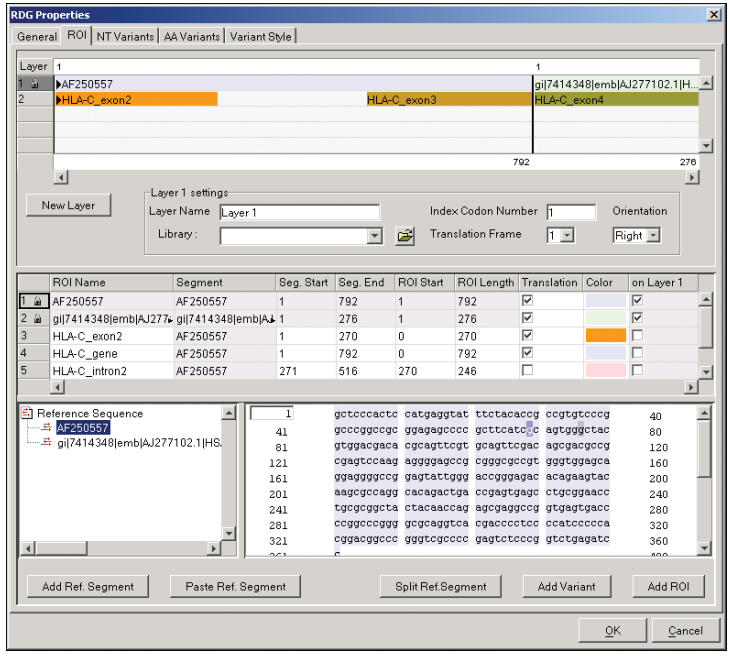
Figure 4-3 NT Variants Tab Showing Table Column Names

- Importing an aligned FASTA file.
- Selecting a sequence within a reference segment, then clicking **Add Variant**. This procedure is described below.

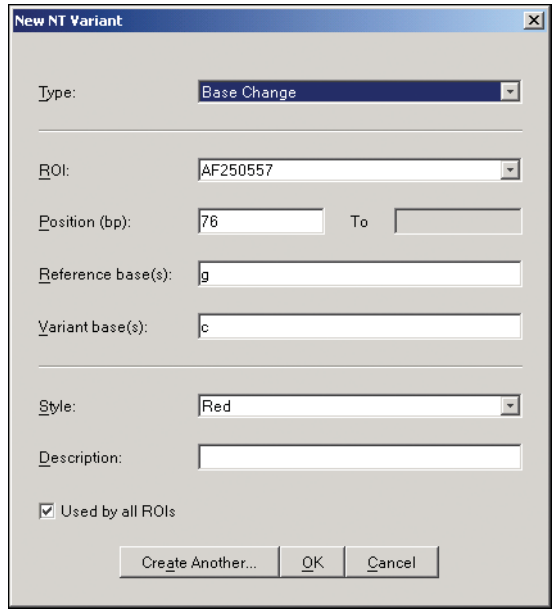
Creating New NT Variants

To create new NT variants:

1. In the SeqScape Manager, select the **Reference Data Group** tab, then click **Properties**.
2. Select the **ROI** tab.
3. Drag to select a sequence in the nucleotide sequence area of the tab, then click **Add Variant**.



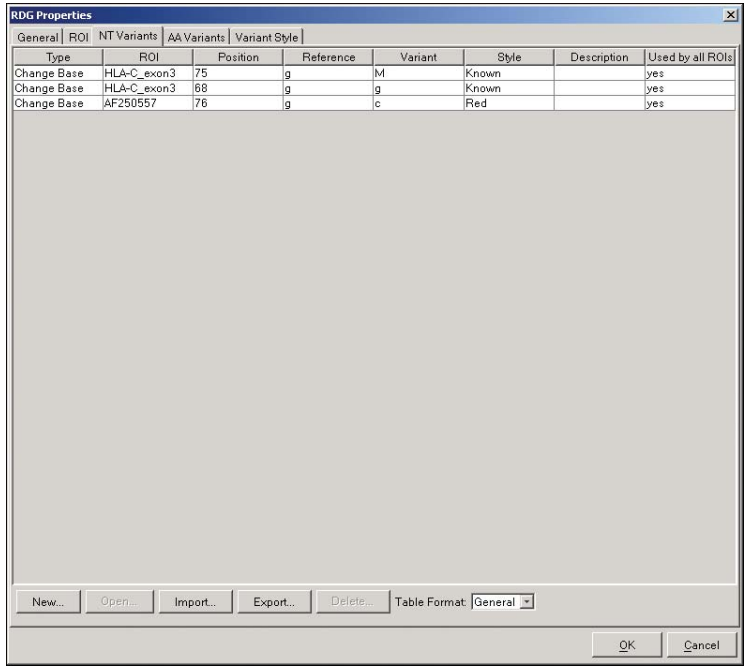
4. In the New NT Variant dialog box, select the type of variant: **Insertion**, **Deletion**, or **Base Change**.



5. Enter the Variant base.
6. If desired, change the style and enter a description.
7. Select the **Used by all ROIs** box if this NT variant is to be used by all ROIs.

8. Click **Create Another**, or **OK** to save the changes.

   After you click OK, the variant additions appear in the list in the NT Variants tab.

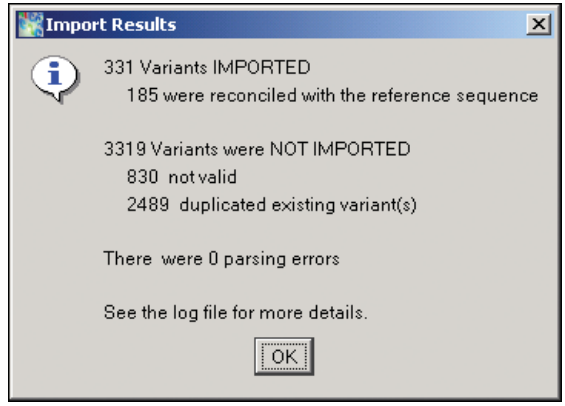


Importing NT Variants in Tab-Delimited Format

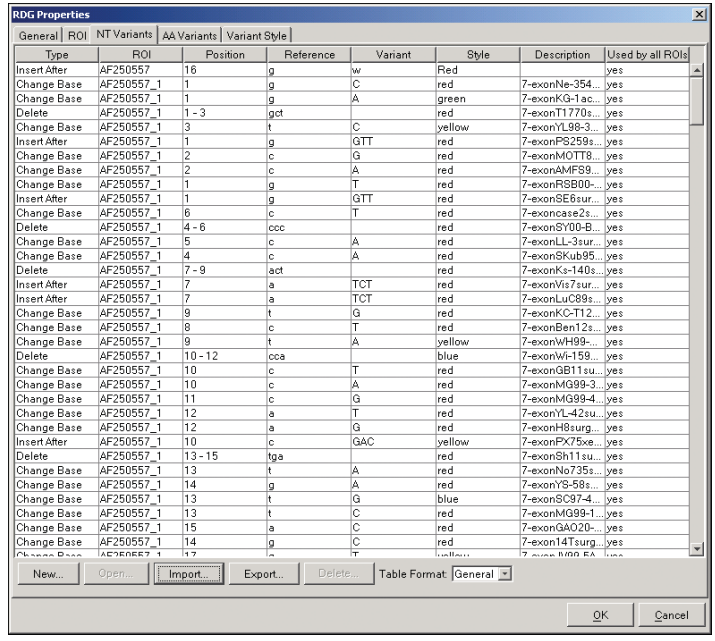
To import an NT variant from a tab-delimited NT variant file:

1. In the SeqScape Manager, click the **Reference Data Group** tab.
2. Select the RDG in the list for which you want to import NT variants.
3. Click **Properties**, then select the **NT Variants** tab.
4. Click **Import**.
5. Navigate to the tab-delimited NT variants file, and click **OK**.

6. An Import Results dialog box opens to show the number of variants imported as shown in the sample below. Click **OK** to close the Import Results dialog box.



7. The new variants appear in the NT Variants list. The Table Format options at the bottom of the window are General (default) and Hugo. If desired, select the format in the drop-down list.



8. Click **OK** to save the imported variants and close the RDG Properties window.

Creating an RDG from Aligned Consensus Sequences

About Creating an RDG

SeqScape software will create a new reference sequence and variants from a set of aligned sequences imported into a blank RDG that contains no reference sequence. The file format of the imported aligned sequences must be in FASTA text. For more information on FASTA format, see Appendix E, "Aligned Variant and FASTA File Format."

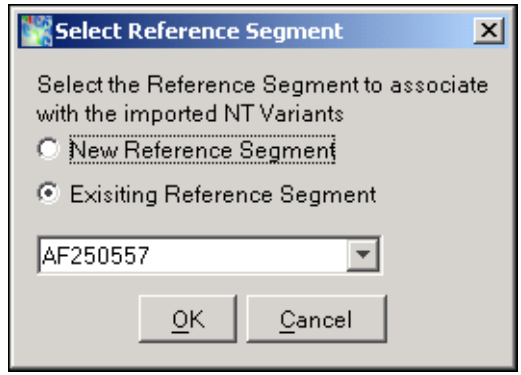
SeqScape software uses the first sequence in the set of aligned sequences in the FASTA file as the reference. The rest of the sequences will be evaluated relative to that first sequence to derive variants. Any positions that are found to differ from the first sequence will be used to populate the variants table.

Importing NT Variants from an Aligned FASTA File

To import NT variants using an aligned FASTA file:

1. Select **Tools > SeqScape Manager**.
2. Click the **Reference Data Group** tab, then select the RDG for which the variant will be added.
3. Click **Properties**.
4. In the RDG Properties window, select the **NT Variants** tab.
5. Click **Import**.
6. In the Import NT Variants dialog box, navigate to and select an aligned sequence FASTA file (.fsta extension).
7. Click **Import**.

8. In the Select Reference Segment dialog box, select the reference segment for which the variants are to be added.

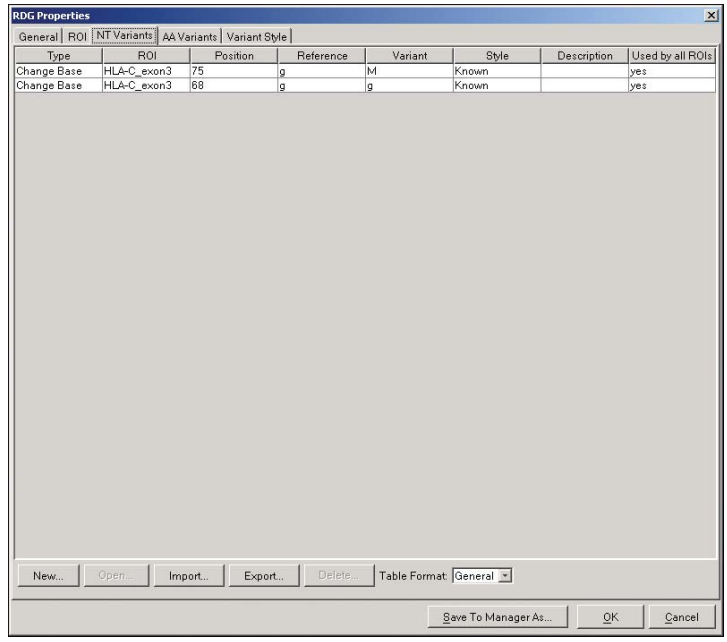


9. Click **OK**.

   After the data is imported, the Import Results dialog box opens, displaying information about the import.

   The first sequence in the imported file will populate the reference. The subsequent sequences will be used to derive variants by comparison to the first sequence. These variants will appear in the Variants table.

10. Click **OK** to close the Import Results dialog box. The list of variants are displayed in the NT Variants tab.



11. Click **OK** to close the RDG Properties window.

Entering New AA Variants

The AA Variants tab lists the known amino acid variants associated with a reference sequence. The entries you define in this tab are used to identify known and unknown amino acid variants in your projects.

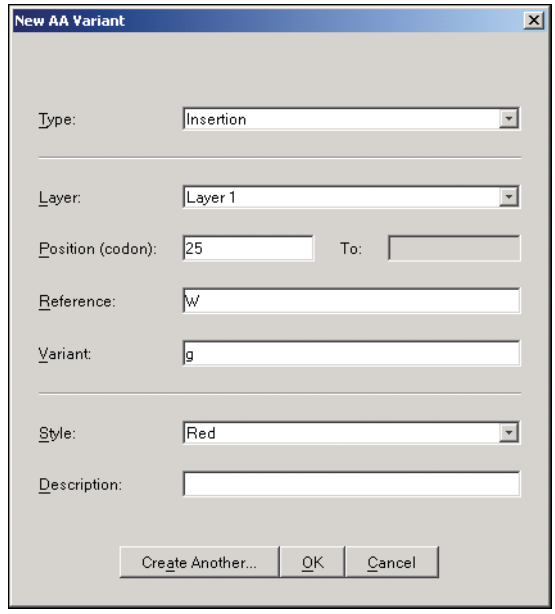
You can enter AA variants in two ways:

- Click Add Variant in the ROI tab, then enter the variant attributes in the New AA Variant dialog box.
- Create a table of variants using Microsoft® Excel, then import the table. The columns in the Excel table must map to the columns in the AA Variants tab. Refer to Figure 4-3 on page 4-29 for the column names.

To enter a new AA variant:

1. In SeqScape Manager, click the **Reference Data Group** tab.
2. Select a listed RDG, and click **Properties**.
3. Select the **AA Variants** tab, then click **New**.

4. Select the type of variant (**Insertion**, **Deletion**, or **Residue Change**).

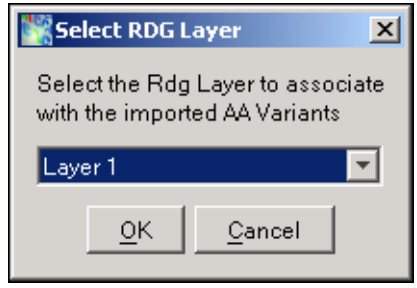


5. Enter the Position (codon) in the reference sequence that you want changed. The Reference appears after you enter the position in the sequence.
6. Enter the variant.
7. Select a color style and enter a description, if desired.
8. Click **OK**. The new variant appears in the AA Variants list.
9. Click **OK** to save the new variant.

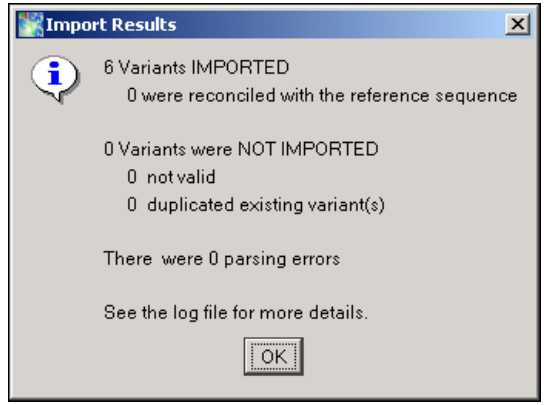
Importing AA Variants

To import an AA variant from a tab-delimited file:

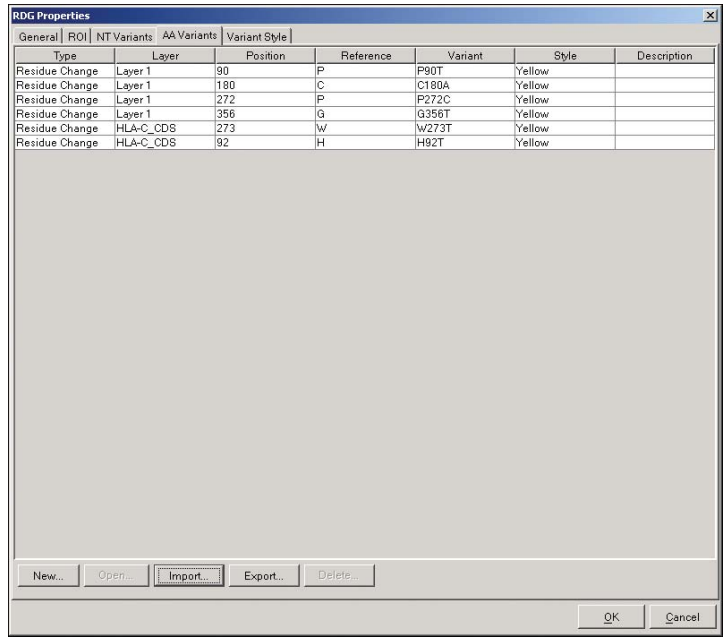
1. In SeqScape Manager, select the **Reference Data Group** tab, then select the RDG you created.
2. Click **Properties**, then select the **AA Variants** tab.
3. Click **Import**, then navigate to the variant data file. It can be a tab-delimited text file (.txt file).
4. Click **Import**.



5. Select any layer from the drop-down list, and click **OK**.
6. Click **OK** in the Import Results dialog box.



The amino acid variants are imported and appear in the list in the AA Variants tab. A sample of AA variants is shown below.



Assigning Styles to Variants

The Variant Styles tab allows you to define text coloring styles that identify different types of variants and change the display characteristics of variants in the Project view. Use the Variant Style tab to assign styles to the variants as desired.

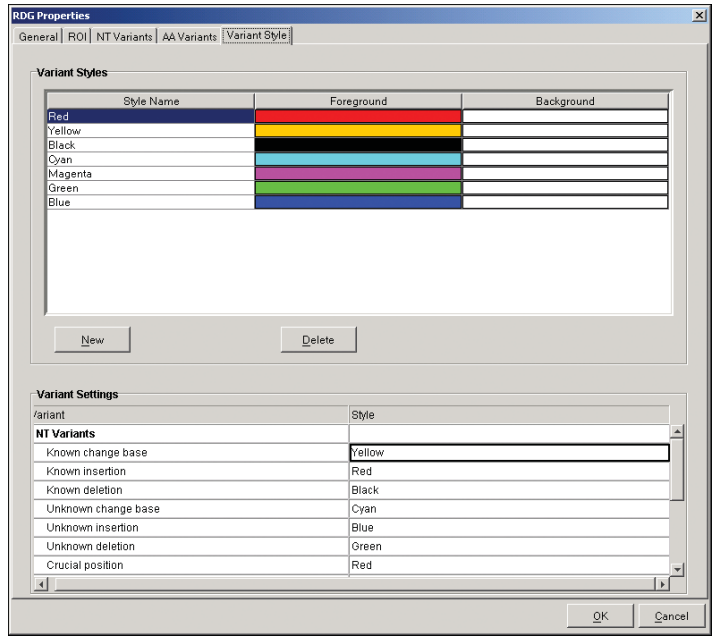
The table at the top of the dialog box displays the generic styles. The table at the bottom of the dialog box lists the different types of variant conditions and their associated styles. The styles you set appear in the Project view to identify the different types of variants.

To assign styles to the variants:

IMPORTANT! When assigning color to text, select light background colors so the text is easy to read.

1. In the RDG Properties dialog box, select the **Variant Style** tab.

   The Variant Styles pane shows the available default colors of the variants.



2. Select the colors in which you want the base changes, insertions, and deletions for known variants displayed.

   a. To add a new color and style, click **New**.

   b. To name the variant style, click the **Foreground Color** box, select a new color in the color palette, then click **OK**.

   c. Select a color from the color palette for the **Background Color**, then click **OK** in both dialog boxes to set the new variant style.

   The variant styles you set appear in the Project view to identify the different types of variants.

3. To delete a color, select the color, then click **Delete**.

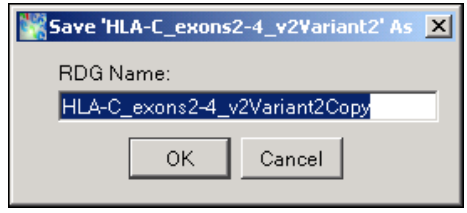
   Note: The first seven Foreground colors cannot be changed or deleted.

4. In the Variant Settings pane, select the colors in which you want the base changes, insertions, and deletions for unknown variants to be displayed. The Variant Styles area shows a list of the available default colors.

Saving a Copy of the RDG

To save a copy of the RDG:

1. In SeqScape Manager, select the RDG you want to save.
2. Click **Save As**.
3. When the confirmation window opens, rename the RDG or click **OK**.

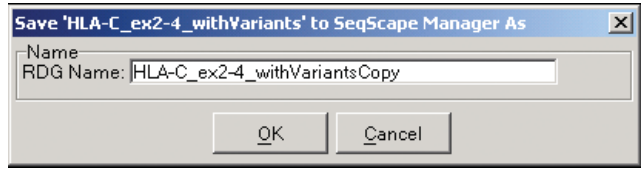


Saving the RDG for Other Projects

If you are working with an RDG that is embedded in a project or project template, you can save a copy of the RDG into SeqScape Manager. This is useful if you make edits to an RDG and want to reuse the RDG for other projects.

To save the RDG:

1. With the project open, in the RDG Properties window, click **Save To Manager As**.
2. Enter a name for the RDG, then click **OK** to save a copy of the RDG under a new name.



If you accept the default name, a copy of the original RDG is saved with that name and is available to use with another project.

Save To Manager As Button

The selections on the Analysis menu, RDG Properties, Analysis Defaults and Display Settings have the Save To Manager As button available for all tabs in each dialog box. The purpose of saving these elements of the project is to have them available to use for changes to the project template for that particular project or other projects that are created.

To use the Save To Manager As button:

1. With the project open, select the **Analysis** menu, then select **RDG Properties**, **Analysis Defaults** or **Display Settings**.
2. Select any tab in any of these three dialog boxes, then click **Save to Manager As**.
3. In the Name field, enter a new name, or accept the default nameCopy and click **OK**.

   The saved copy is available to import into another project.

Creating a Project Template 5

This chapter contains:

Workflow for This Chapter

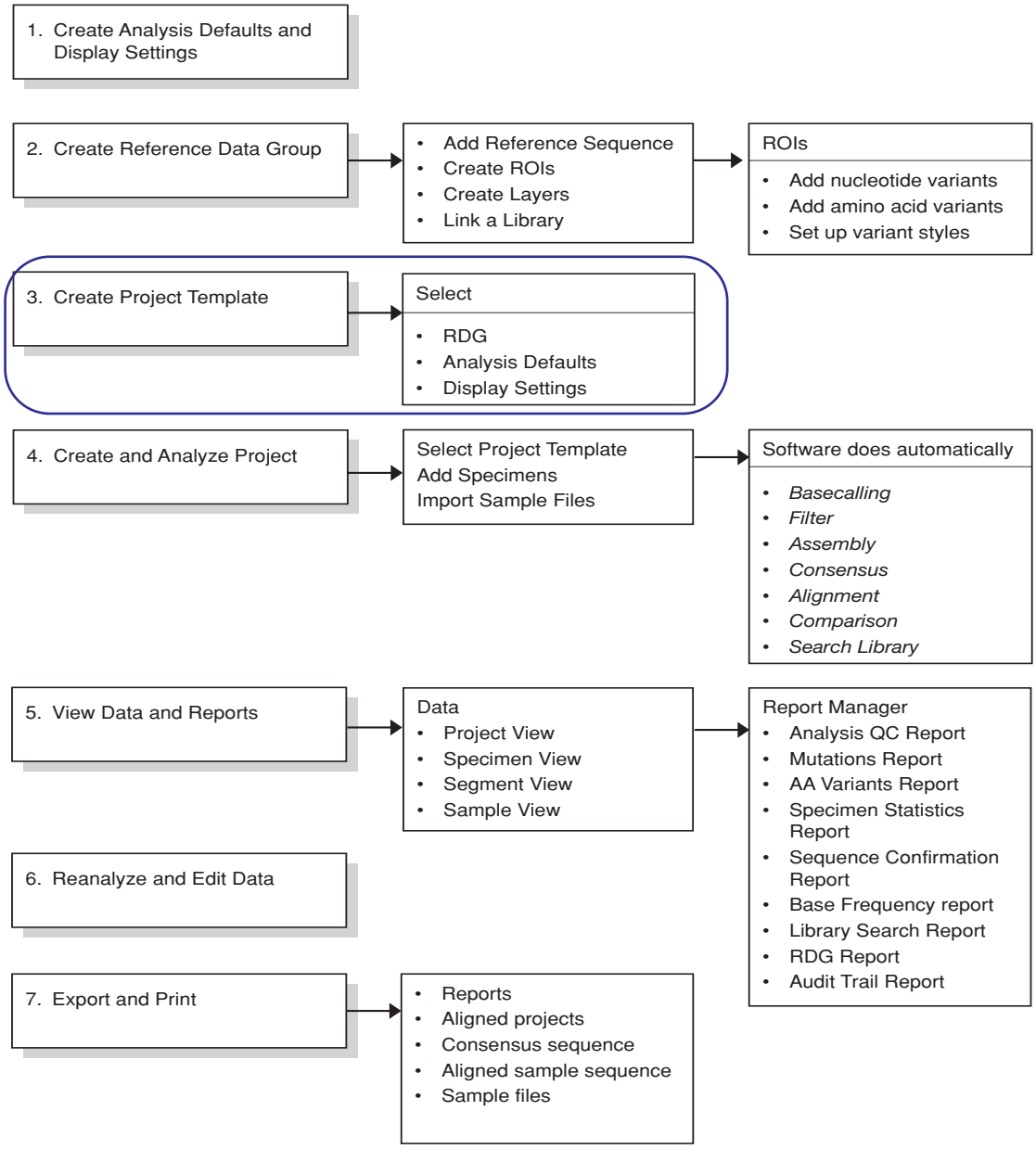


Figure 5-1 Showing Create Project Template Step

Creating a Project Template

Before you can effectively use the ABI PRISM® SeqScape® Software Version 2.0, you must create and configure a project template. A project template contains all the reference data and settings needed to analyze your data automatically. It defines how the software analyzes and displays your samples. When project templates are created in the SeqScape Manager, they can be imported, exported, and edited.

About Creating a New Project Template

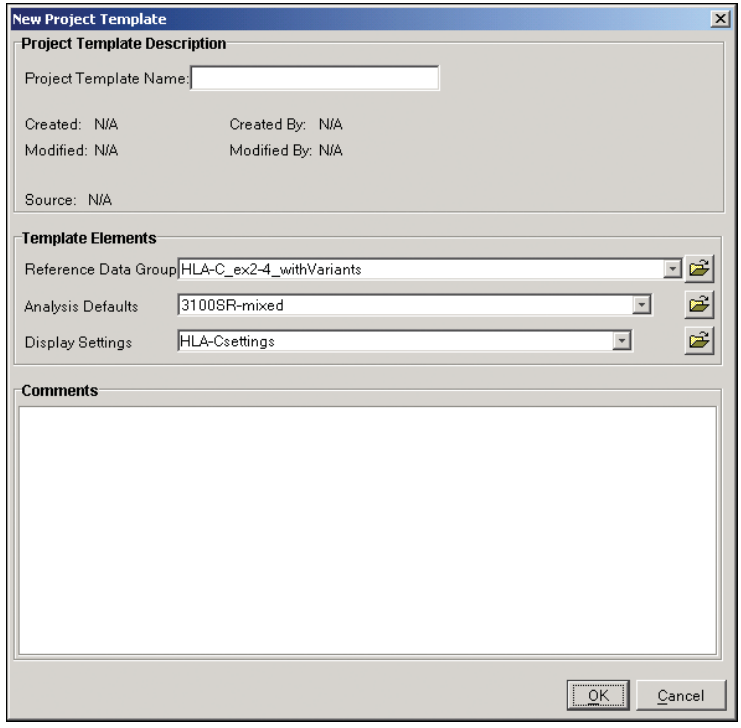
When you create a new project template from the SeqScape Manager, you select:

- A Reference Data Group – Reference sequence and associated data to which all the specimens in a project are compared. See "Creating a New RDG Using SeqScape Manager" on page 4-12 for more information.
- Analysis defaults – Settings that are used to analyze the data. See "Specifying the Analysis Settings" on page 3-11 for more information.
- Display settings – Settings that are used to display the data. See "Specifying Display Settings" on page 3-16 for more information.

Creating a New Project Template

To create a new project template:

1. In the SeqScape window, select **Tools > SeqScape Manager**.
2. Select the **Project Templates** tab, then click **New**.



3. Enter a name for the project in the Project Template Name field.

   Note: The project template name must contain only characters that conform to the Windows file system. Refer to "File-Naming Convention" on page 2-9 for a list of all invalid characters.

4. Select the desired Template Elements from the drop-down lists, then click **OK**.

Saving Project Components

About Saving Template Components

If you modify RDG, analysis, or display settings within a project, the changes are valid only in that one project. However, if you want to save those settings so they can be applied to other projects, you can create new SeqScape Manager template components based on existing template components.

Saving Template Components from Within a Project

To save project template components:

1. Within a project, select the Analysis menu, then select one of the template components that you want to modify:
   - **RDG Properties**
   - **Analysis Defaults**
   - **Display Settings**
2. Make the desired modifications to the component.
3. Click **Save To Manager As**.

   An appended name of the current template component appears in the Save.xx to the SeqScape Manager As dialog box.
4. Leave the name unchanged or change it.

   IMPORTANT! You cannot save over an existing template component. You must delete the existing master component from the SeqScape Manager before you can save a new template component.
5. Click **OK**.
6. To use the modified component for other projects, make a new project template that uses the new components.

Examples of Changing the Settings Within a Project

Example 1

1. Create a project template and apply it to a project.
2. Analysis > Analysis Settings, then change a sample analysis setting.

   The underlying Analysis Defaults are unchanged in the SeqScape Manager.

Example 2

1. Create a project template and apply it to a project.
2. Modify each component of the template.
3. Change a variant style in the RDG, then select **Save To Manager As**.

   A new RDG in the SeqScape Manager reflects this change, but the old RDG in the SeqScape Manager remains unchanged. Therefore, the project template using the old RDG is also unchanged.

Note: In both examples, the open project displays the changes.

Creating and Analyzing a Project

6

This chapter contains:

Workflow for This Chapter

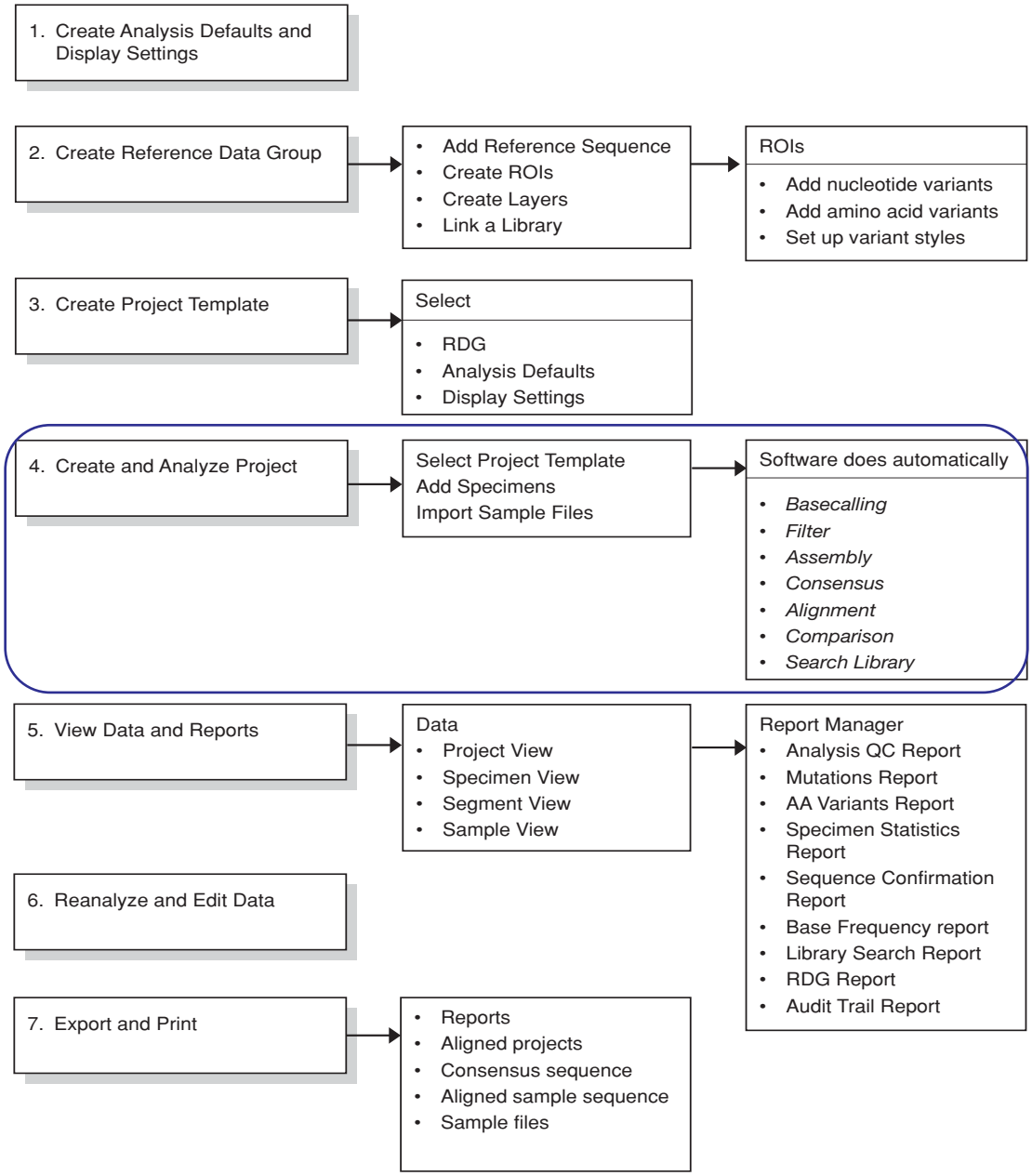


Figure 6-1 Showing Create and Analyze Project Steps

Before You Begin Creating a Project

Before you can create a project in the ABI PRISM® SeqScape® Software Version 2.0, you must have created a project template that contains:

- A Reference Data Group
- Analysis Defaults
- Display Settings

What an Analysis Entails

The analysis in the SeqScape software:

- Basecalls the raw data
- Assigns quality values and identifies mixed bases
- Filters out poor quality data and excludes that data from further analysis
- Assembles samples within each specimen to generate a consensus sequence
- Aligns each specimen consensus sequence to the reference sequence
- Compares the aligned consensus sequence to the reference sequence
- Displays analysis results

Ways to Create and Analyze a New Project

After the analysis defaults are set up, you can create a new project for data analysis by:

- Using the New Project Wizard (see page 6-5)
- Using an existing project template (see page 6-10)

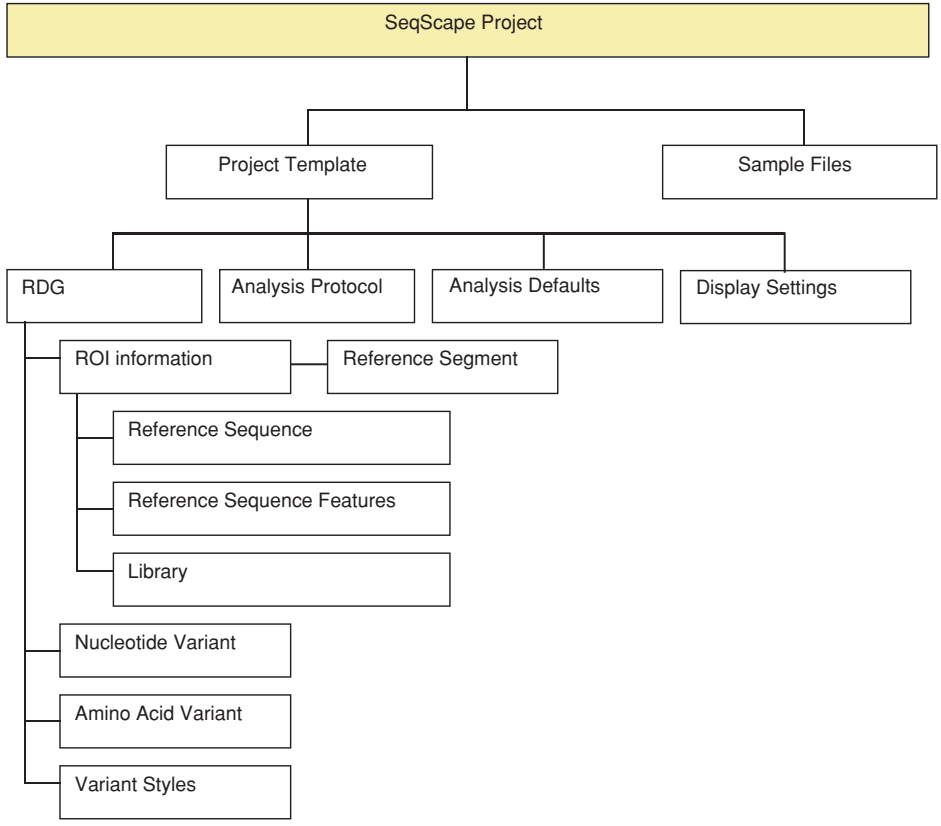


Figure 6-2 Components of a Project

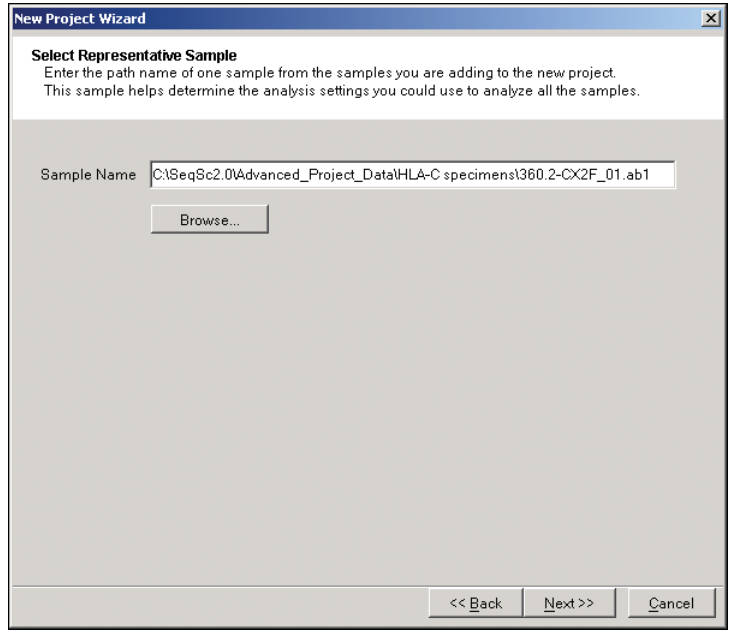
Using the New Project Wizard to Create and Analyze a Project

The New Project Wizard

The New Project Wizard takes you through the process of setting up a new project.

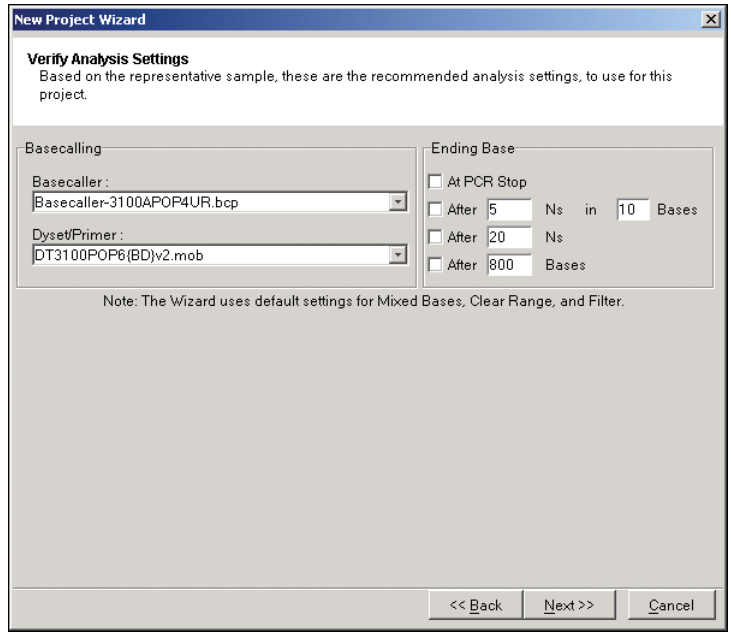
To create a new project using the New Project wizard:

1. Launch the SeqScape software.
2. Select **File** > **New Project Wizard**.
3. Enter a name for the new project in the Project Name field, then click **Next**.
4. Enter a sample name, or click **Browse** and navigate to the sample you want.



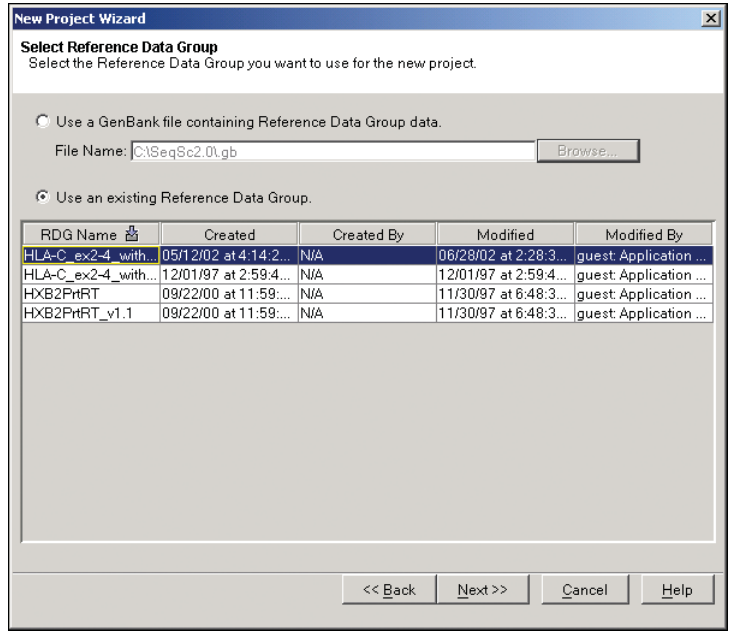
5. Select a sample with the .ab1 extension, then click **Open**.

6. Click **Next**. The Wizard uses analysis settings based on your sample choice.



7. Verify the analysis settings (Basecaller, DyeSet/Primer files, and Editing Bases), then click **Next**.

8. In the Select Reference Data Group page:

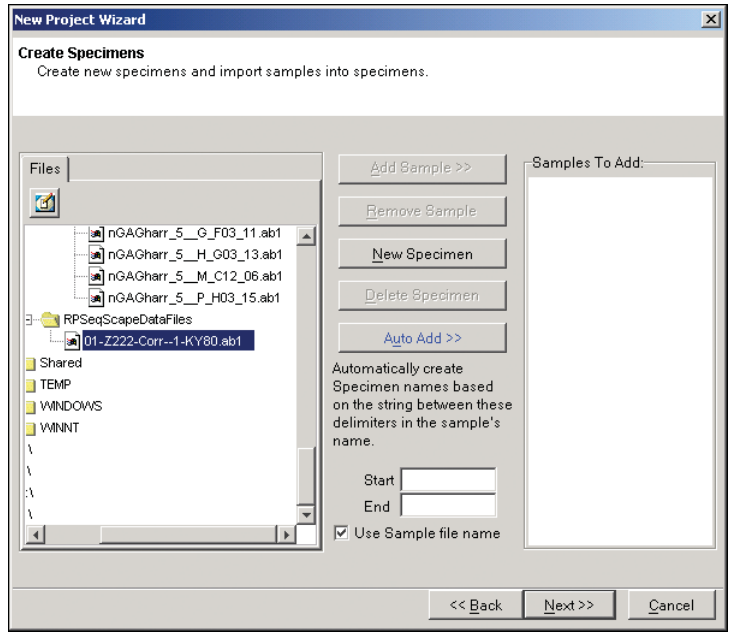


a. Do one of the following:

- Select **Use a GenBank file containing Reference Data Group data**, then specify a GenBank file. Or,
- Select **Use an existing Reference Data Group**, then select a Reference Data Group file in the list.

b. Click **Next**.

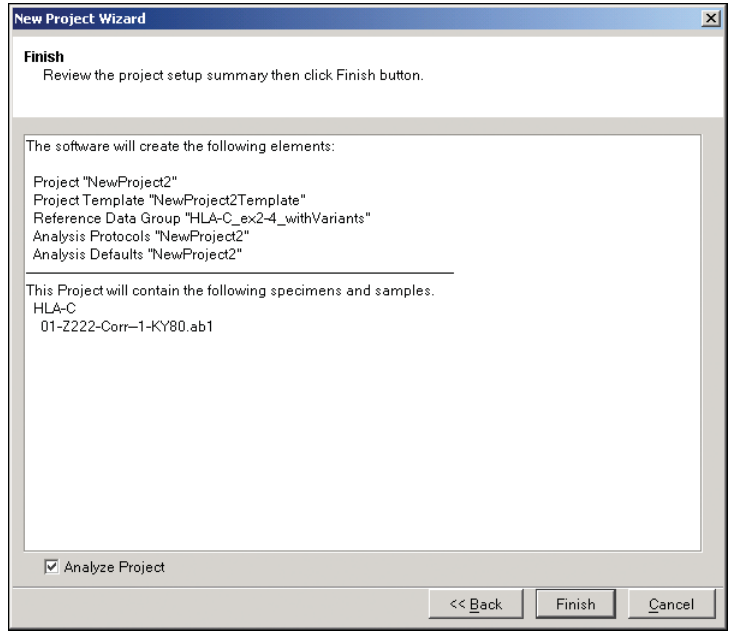
9. Add specimens and import samples in the Create Specimens page:



a. In the Files section, select a sample, multiple samples, or a folder, then click **Auto Add**.

b. Click **Next**.

Note: For information on adding specimens, see "Adding Specimens and Importing Data into a Project" on page 6-11.



When you use the Project Wizard for the first time, master display settings are created. These same settings are used if the wizard is used again.

10. Review the setup. Click **Back** to change the setup, if necessary.
11. Do one of the following:

To analyze …	Then …
Now	Click **Finish**.
Later	1. Deselect **Analyze Project** at the bottom left corner of the page. 2. Click **Finish**.

12. When you close the new project, click **Yes** to save it.

This project is now available in the list of available projects in the SeqScape Manager.

Creating and Analyzing a New Project Using a Project Template

You can use an existing project template to create a new project. A project template contains:

- A Reference Data Group (RDG)
- Analysis defaults
- Display settings

About the Project Template

For convenience, one example project template is included in the SeqScape software. To create your own project template, see "Creating a Project Template" on page 5-3.

Table 6-1 Components of the Project Template Included in SeqScape Software:

Template Component	File Name
Project Template Name	HLA-3100_v2
Reference Data Group	HLA-C_exons2-4_v2
Analysis Defaults	3100SR-mixed_v2
Display Settings	DefaultDisplaySettings_v2

Creating a New Project Using a Template

To create a new project using a project template:

1. Select **File** > **New Project**.
2. When the New Project window opens, select a template from the list and enter a project name.

   Note: To see the whole name in the list, click-drag the Project Template heading to the right when the double-headed arrow cursor appears on the column bar.
3. Click **New**.

   The new project using the selected template opens.

Adding Specimens and Importing Data into a Project

Overview

All sample data from a single biological source should be placed inside a specimen within a project. All sample data inside a specimen is assembled, and a consensus sequence is produced. You can think of each specimen as holding the assembled samples from one PCR product, for example. The consensus that is generated is compared to the references and aligned to the other consensus sequences from other specimens.

If you have new, unanalyzed data, you need to create specimens in the project to hold the data. You can add specimens to a project automatically or manually.

For more information on what types of data can be imported into a project, see "Adding Specimens and Importing Samples Manually" on page 6-14.

IMPORTANT! Unanalyzed specimen and sample data show a red slash line through their icons, indicating that analysis is needed.

IMPORTANT! Specimen names can be edited only after they are imported. Sample names cannot be edited from within SeqScape software at any time.

Adding Specimens and Importing Samples Automatically

Using a text delimiter, SeqScape software simultaneously and automatically creates specimens and imports unanalyzed or analyzed samples into a project.

Sample IDs and Sample Names

To take advantage of this feature, your sample ID (which is created by the data collection software and stored within each sample file) needs to contain the same prefix for all samples in each specimen.

IMPORTANT! The sample ID is the name that you assign to the sample in the data collection software. You cannot modify the name.

The sample file name is longer than the sample ID, and often is derived from the sample ID. The sample file name is what you see when looking for the sample.

The text delimiter is chosen from the sample ID. Using this function, a set of sample files that are grouped into the same folder and that share a similar delimiter can be imported into their corresponding specimens in a single step.

In the example shown in Figure 6-3, the delimiter is a dash. Everything to the left of the delimiter determines the specimen name. When you select Add Automatically, the sample files are automatically transferred into specimens that are also created and named automatically. In this example, the first specimen includes all files that start with A1.

The sample ID also appears in the Annotation view of the sample, as indicated in Figure 6-3.

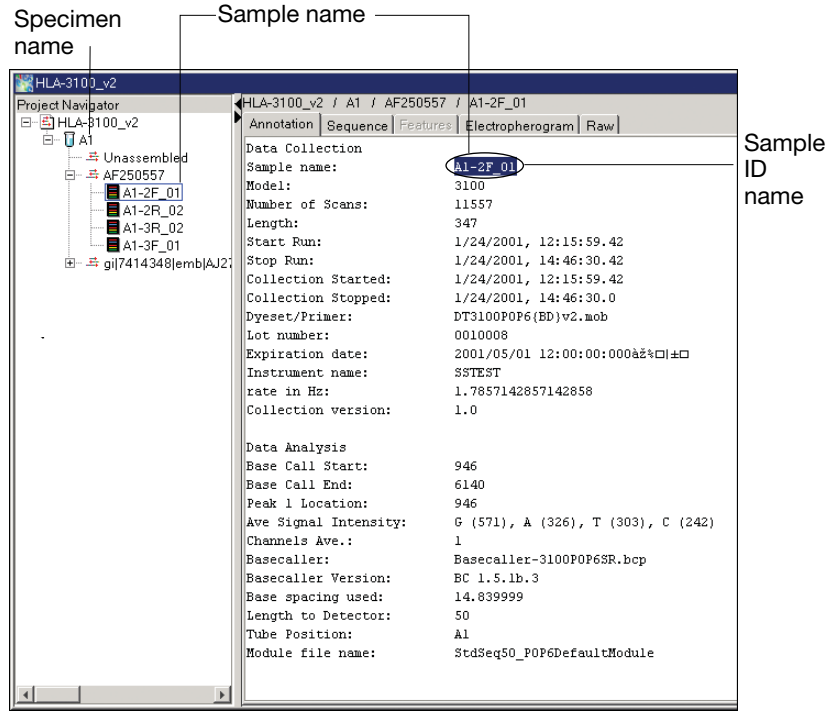


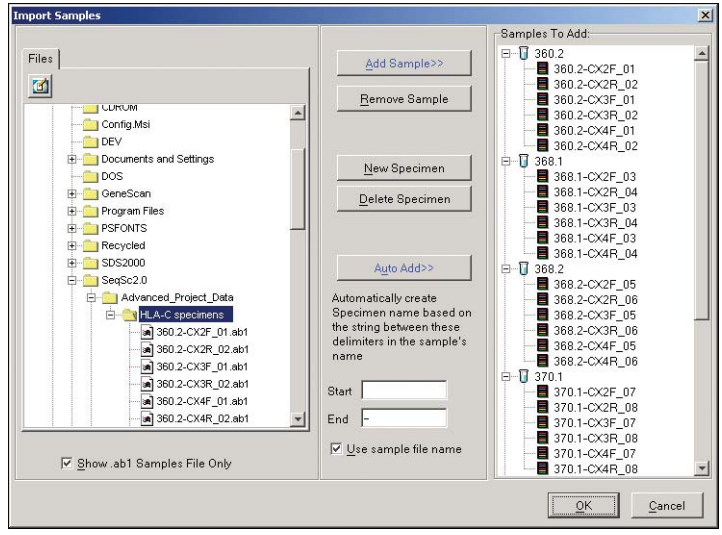
Figure 6-3 Annotation Tab

Creating a Specimen Automatically

To create a specimen and import samples automatically:

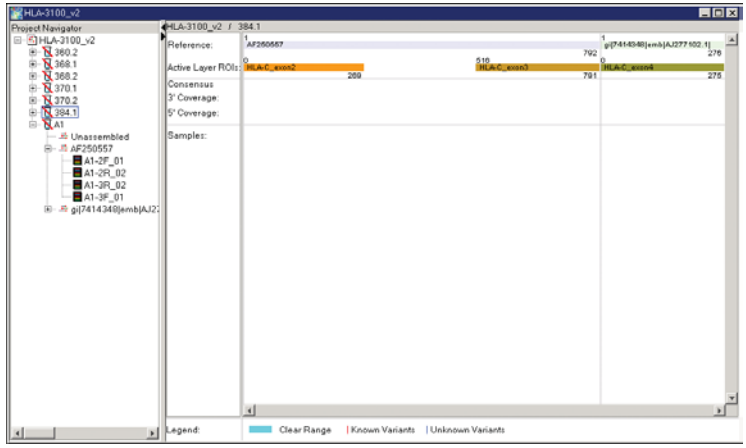
1. With the Project window open, select **File > Import Samples To Project** or click  .
2. In the Specimen name delimiter field, enter the delimiter text.

   Note: The delimiter text is derived from the sample ID name in the data collection software sample sheet or plate record. In the figure in step 3, the delimiter is a dash. The sample ID name from the data collection software appears in the Sample name section of the Annotation view of the sample.
3. Select the folder containing the samples to be imported, then click **Auto Add**.



Based on the text delimiter, the samples are automatically imported into the appropriate specimens (in this example, the specimens are shown under HLA-C specimens).

4. Click **OK** to import the specimens and samples into the project.



Adding Specimens and Importing Samples Manually

You can import the following types of sample data into specimens within a project:

- Sample data files from ABI PRISM instruments
- Database files
- Specimen text-only files

Table 6-2 Types of Sample Data

To import …	See …
Sample data files	“Adding Specimens and Importing Data Files” on page 6-15
Database files	“Importing Samples from a Database” on page 6-20
Specimen text-only files	“Importing Text-Only Files” on page 6-22

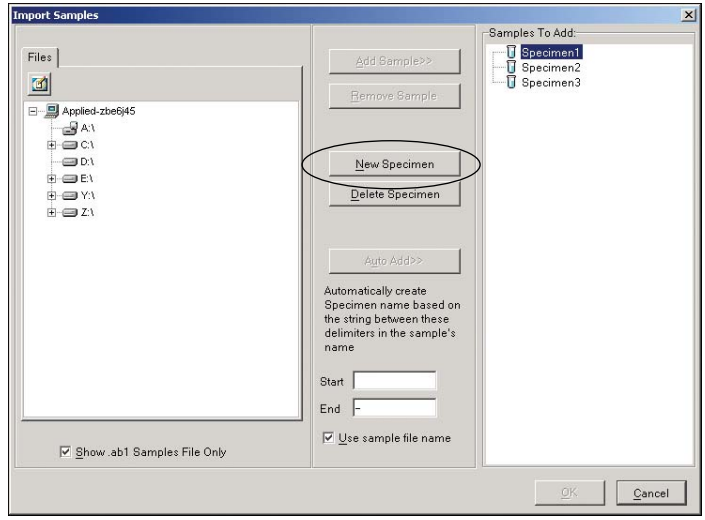
Adding Specimens and Importing Data Files

To import unanalyzed or analyzed sample data, the files must be in ABI format. Sample data is imported into specimens in the project. New specimens are created in the Import Samples dialog box.

To add specimens and import sample data files:

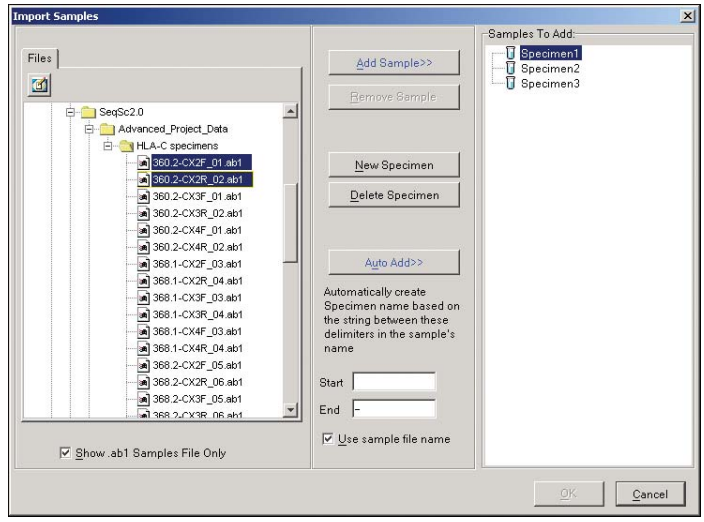
1. With the Project window open, select **File > Import Samples To Project**, or click  to open the Import Samples dialog box.

2. Create a new specimen:
   a. Click **New Specimen**.
   b. Add two more specimens.



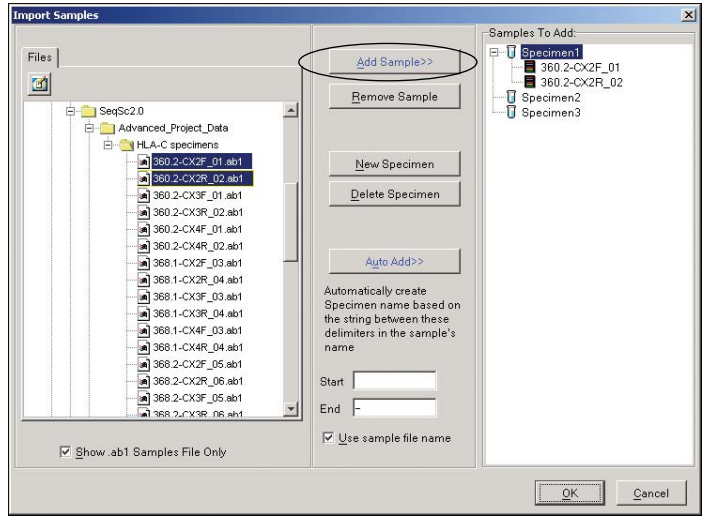
3. In the Samples To Add section on the right, select the specimen into which to import the data.

4. In the Files pane, navigate to the samples you want to add.
5. Select the first specimen in the Samples to Add pane.



6. In the Files section, select the sample data files.

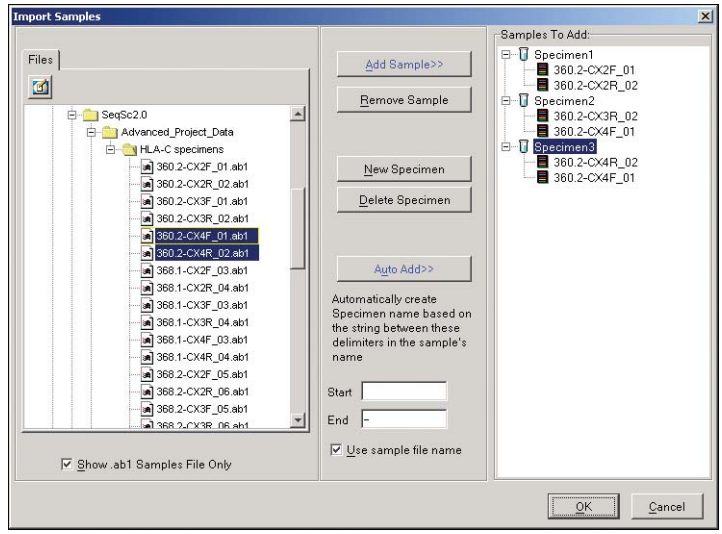
To import …	**Then …**
A single sample	Select the single sample.
Multiple samples	**Ctrl+Click** to select contiguous or noncontiguous samples.
All samples in a folder	Select the folder.



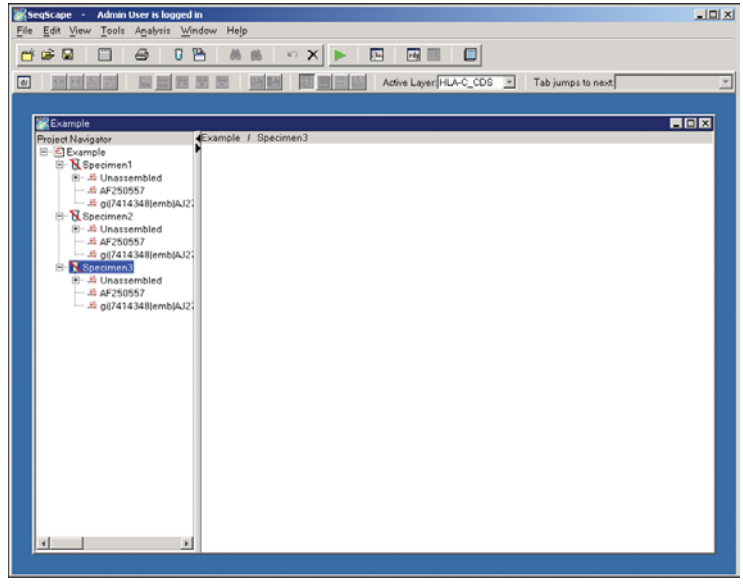
7. Click **Add Sample**.

   The sample data appears in the selected specimen, showing where the data will be imported. No data is actually imported into the project until you click OK.

8. Select the second specimen, select the samples, and click **Add Sample**. Repeat this for the third specimen.

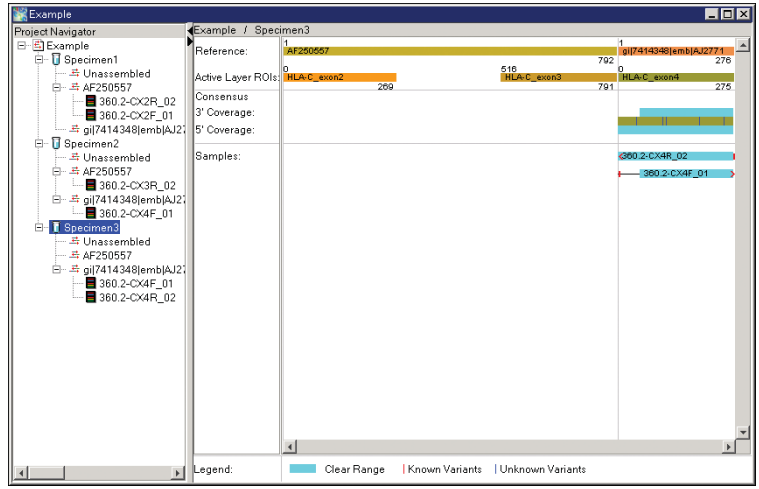


9. Click **OK** to perform the imports and return to the Project window. The project reflects the new specimens and samples, with the specimens shown with a red line through them. This indicates that the samples are unanalyzed and unassembled.



10. If desired, select each specimen and type a new name for the specimen, then press the **Enter** key.

11. The green arrow button  on the toolbar indicates that the samples need to be analyzed. Click this button. After the samples are analyzed, the red line through the specimen is gone and the samples are assembled as shown in the figure below.



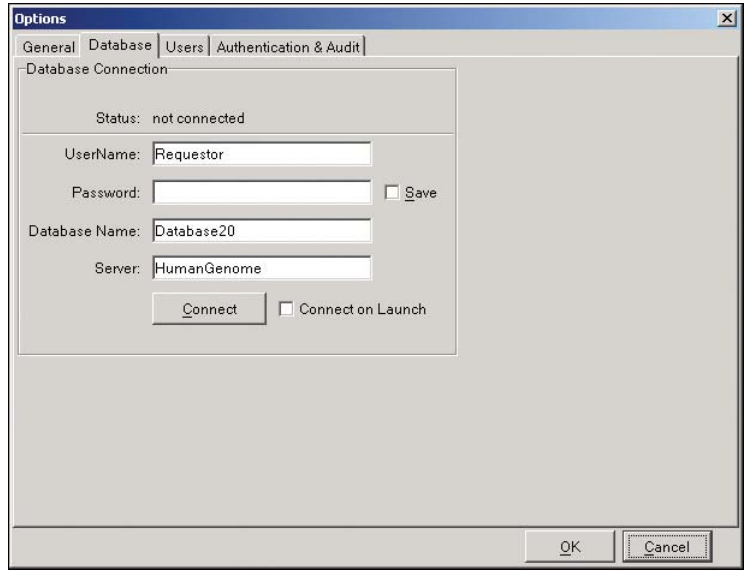
12. Close the project and save it.

Importing Samples from a Database

You can also import ABI PRISM sample data stored in a database if you have Sequence Collector v3.0 installed on your computer.

To import sample data using Sequence Collector software:

1. Select **Tools** > **Options**.
2. Select the **Database** tab.



3. Enter the appropriate information in the UserName, Password, Database Name, and Server fields.

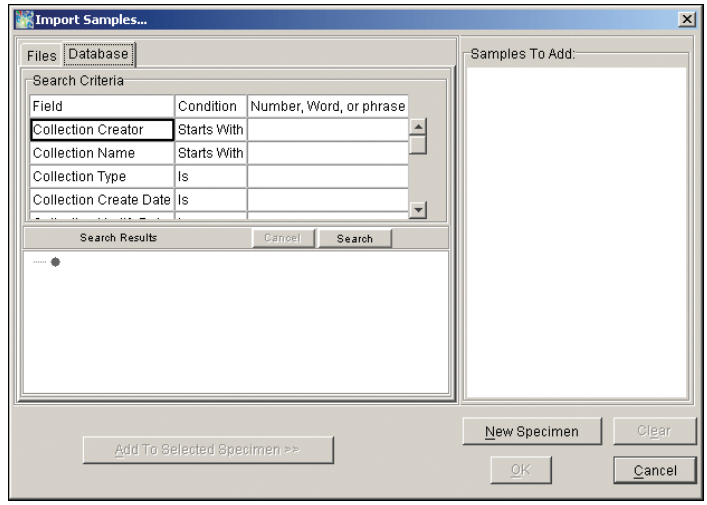
   Note: These names are the same as those used for Sequence Collector.

4. Click **Connect** to start the connection.

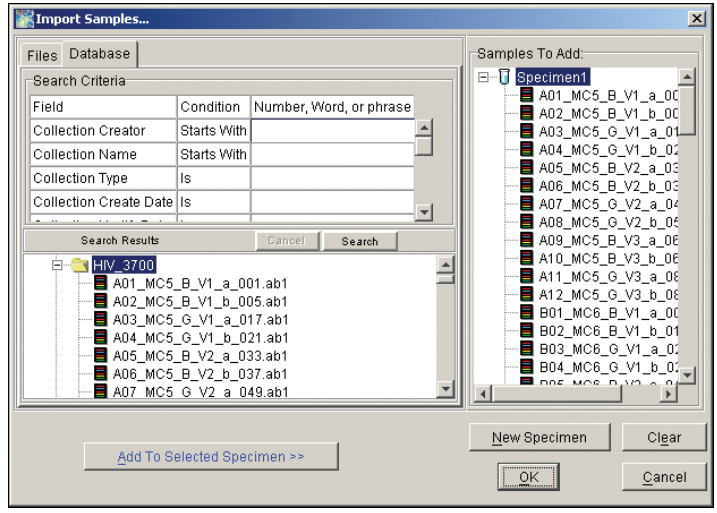
   When a connection is made, the Status displays as "connected" and a Disconnect button is displayed.

5. Click **OK**.
6. Create or open a project.
7. Select **File > Import Samples To Project**.

8. Select the **Database** tab to search for your samples. The interface is the same as that in Sequence Collector.



9. Locate the samples, then import them as you would any other sample files. Select the files on the left side, select the target specimen on the right side, then click **Add To Selected Specimen**.

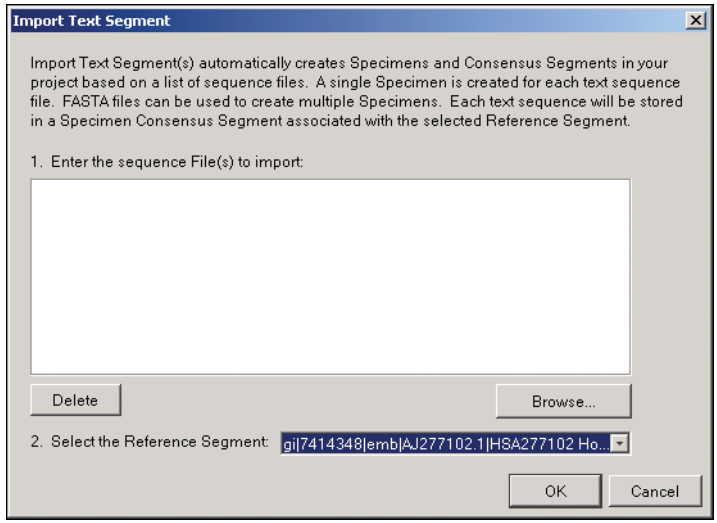


Importing Text-Only Files

You can import into a project a consensus sequence in text format as a text-only specimen.

To import text or previously assembled sequences:

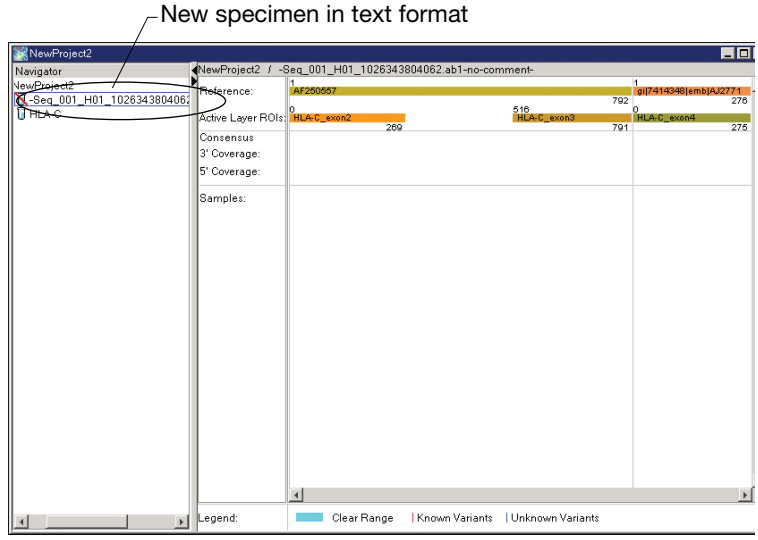
1. In the Project Navigator, select the project name, then select **File > Import Text Segment**.



2. Click **Browse** and navigate to the target segment.
3. In the Import Text-Only Segment dialog box, select the text file (.fsta format), then click **Import**. The segment appears in the previously blank section in the Import Text Segment dialog box.
4. Repeat steps 2 and 3 to add additional text segments.

5. Click **OK**.

   A new specimen is created with the name specified in the first line of the file.



Removing Samples or Specimens

To remove samples or a specimen from a project:

1. In the Project Navigator of the project, select the samples or specimen you want to remove from the project.
2. Press the **Delete** key.

   IMPORTANT! This deletes the results and cannot be undone. If you press Delete in error, close the project without saving to restore the results.

3. In the Confirm Delete dialog box, click **Yes**.

Analyzing the Data

After you import all your data, you can run the analysis. After new data is imported or analysis settings are changed for a sample, the Analyze icon in the toolbar appears green, indicating that there is unanalyzed data.

Running an Analysis

To run an analysis in the project, click (Analyze), or select **Analysis > Analyze**.

Reanalyzing a Project Using a Different Project Template

When You Would Want to Do This

After you analyze an entire project that contains many samples, you may want to reanalyze all the data using a project template that contains different settings or reference data.

Saving a Project Before Reanalyzing

IMPORTANT! Applying a new project template discards all analyzed data, including basecalls, features, alignments, and manual edits. To avoid discarding the data, rename the project to keep your original analysis, if desired.

To save a project that you want to reanalyze:

1. Select **Tools** > **SeqScape Manager**.
2. In the Project list, select the project that you want to save before reanalyzing.
3. Click **Save As** and rename the project.
4. Click **OK**.

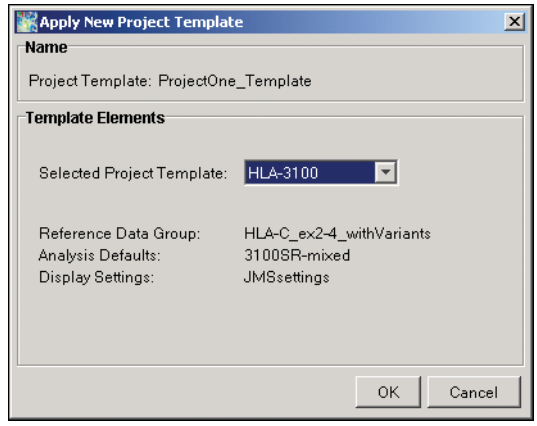
   The project is saved under a new name and your original project remains in the list.

Applying a Template to an Existing Project

To reanalyze the project with a different template:

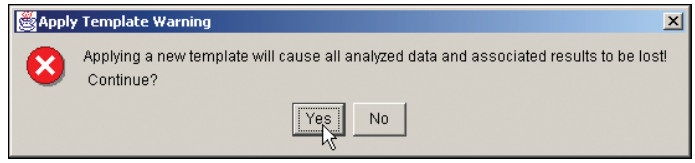
1. Create a template containing the desired changed settings and/or reference sequence (see "Creating a New Project Using a Template" on page 6-10).
2. Open the existing project that has the data analyzed using the old settings.

   IMPORTANT! Save the project under a new name if you want to keep the current project data to compare to the new project data. If you do not save the project, all the data is overwritten when you apply a new project template.
3. Select **Analysis > Apply Project Template** to open the Apply New Project Template dialog box.



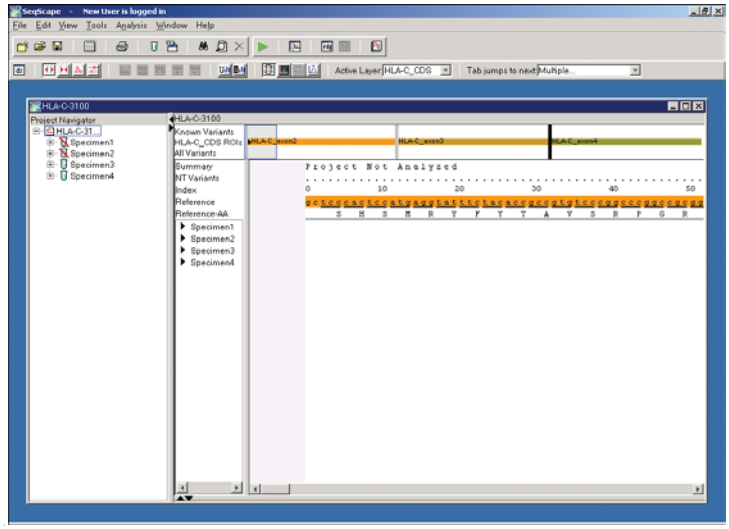
4. In the Selected Project Template drop-down list, select the project template that you want to apply to the project.
5. Click **OK**.

6. A dialog box opens warning you that all analyzed data and results will be discarded. To continue, click **Yes**.



The project opens, containing all the specimens and samples, but the data is unanalyzed.

7. To analyze the data with the new template, select **Analysis > Analyze**.



Incorporating Variants into the Project RDG

About Incorporating Variant Sequences

You can incorporate variants into an active project RDG by doing one of the following:

- Changing an unknown variant in a specimen to a known variant
- Adding a variant
- Importing a file containing variant sequences
- Importing a set of variants from a TXT file

Note: If you have a master RDG and want to include additional variants in the RDG, you must incorporate them using the SeqScape Manager.

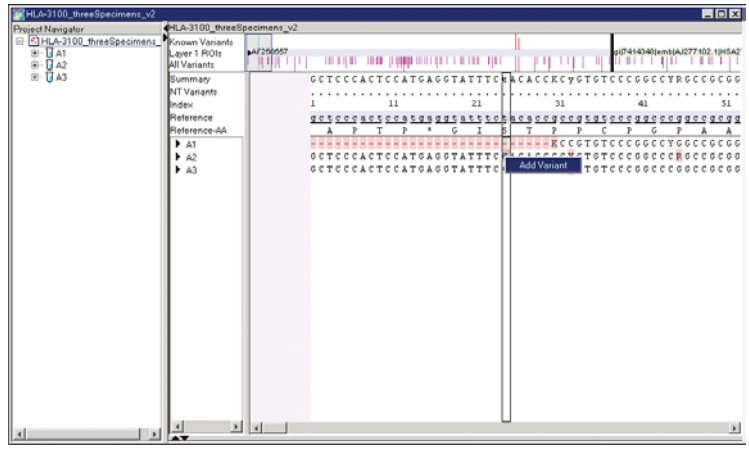
You can incorporate variants automatically by importing a file of a tab-delimited text file of variant positions and descriptions. By this method, variants are created and styles are applied to all the variants in the file.

Alternatively, you can change an unknown variant in a specimen to a known variant, or you can create variants by adding them to the Reference Data Group.

Changing a Single Unknown Variant to a Known Variant

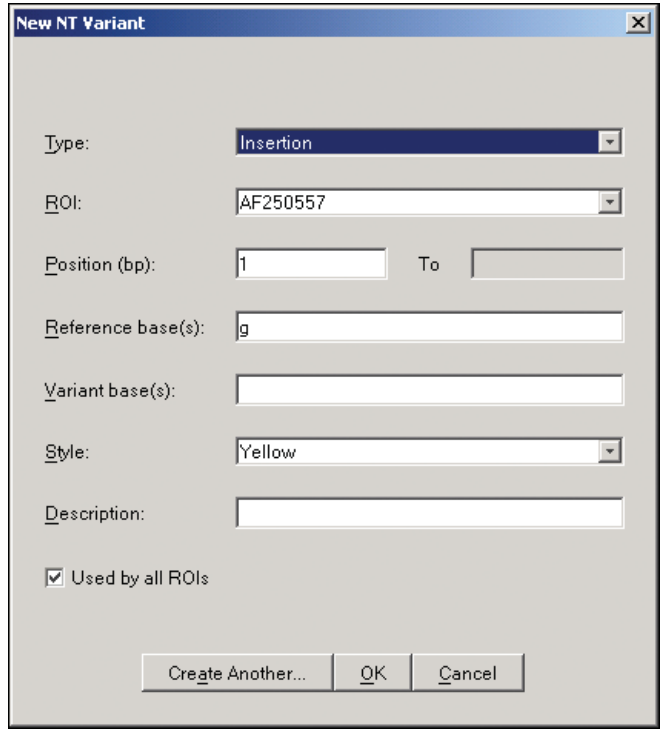
To change an unknown variant to a known variant:

1. Select a variant base in a specimen, then right-click it.



2. Select **Add Variant** from the shortcut menu to open the New NT Variant dialog box.

   The information regarding the type and position of the variant appears in the New NT Variant dialog box.



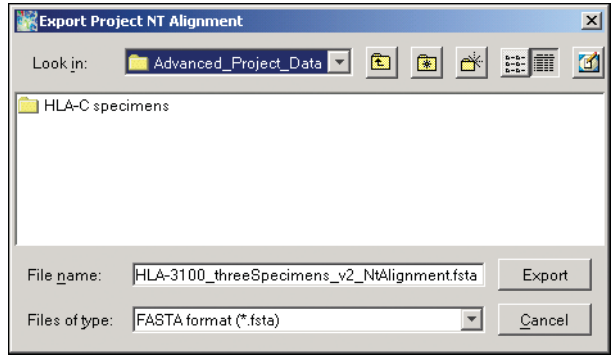
3. Select a variant style from the Style drop-down list.
4. In the Description field, enter text, if desired, then click **OK**.
5. Repeat steps 1 through 4 for another variant.

Changing Multiple Unknown Variants

To change multiple unknown variants to known variants, you need to export unknown variants in a project alignment file and then import them into the project.

To change multiple unknown variants to known variants:

1. Open the project and select the specimen containing the unknown variants.
2. Select **File > Export > Project Alignment-Nucleotides**.

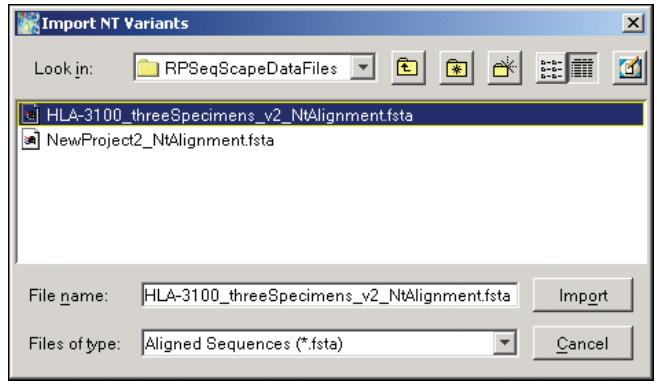


3. In the Export Project NT Alignment dialog box, select a destination for the exported data, then click **Export**.

Importing Variants

To import variants:

1. Select **Analysis > RDG Properties**.
2. Select the **NT Variants** tab.
3. Select **Import**.
4. In the **Import NT Variants** dialog box, navigate to the project alignment file, then select it. Make sure the Files of type is set to **All Files** or **Aligned Sequences**.



5. Navigate to or select the file to import.
6. Click **Import**.
7. Select the reference segment in the drop-down list in the Select Reference Segment dialog box, then click **OK**.

   The variants appear in the NT Variants table as Known variants. The descriptions are the specimens in which the variants appear and the style is the default style for the variant type.
8. Select the **Variant Style** tab in the RDG Properties dialog box to change the default style in the RDG and enter a description of the imported variants.
9. Select the **NT Variants** tab to be sure the variants are Known.
10. Click **OK** to save the variants.

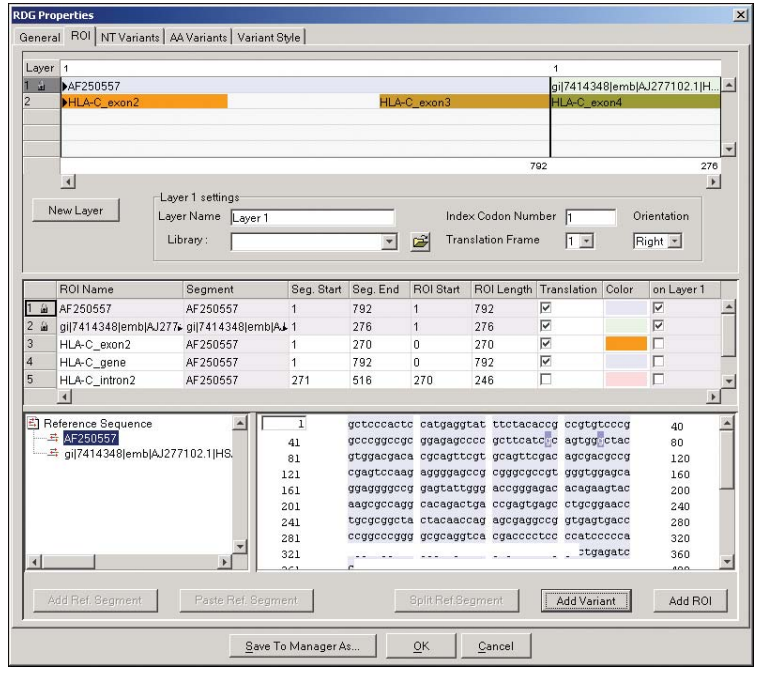
Creating a New Variant in a Project

You can add a variant to a project by:

- Entering the type and position of the variant in the Variants tab of the RDG Properties dialog box.
- Selecting the location on the reference sequence in the Sequence tab in the RDG Properties dialog box. The appropriate information regarding the variant is automatically entered in the variant dialog box.

To create a new variant in the project:

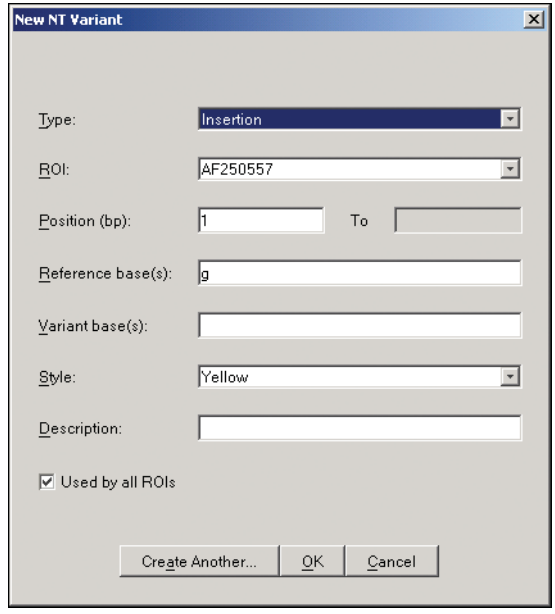
1. From the Project window, open the RDG Properties dialog box by selecting **Analysis > RDG Properties**.
2. Click the **ROI** tab, then select **Add Variant**.



3. In the New NT Variant dialog box, select the type of variant (**Base Change**, **Insertion**, or **Deletion**).

4. Select the Position and either **To** (position) or **Variant base**.

   Note: The **Reference** base is entered by the software based on the position.

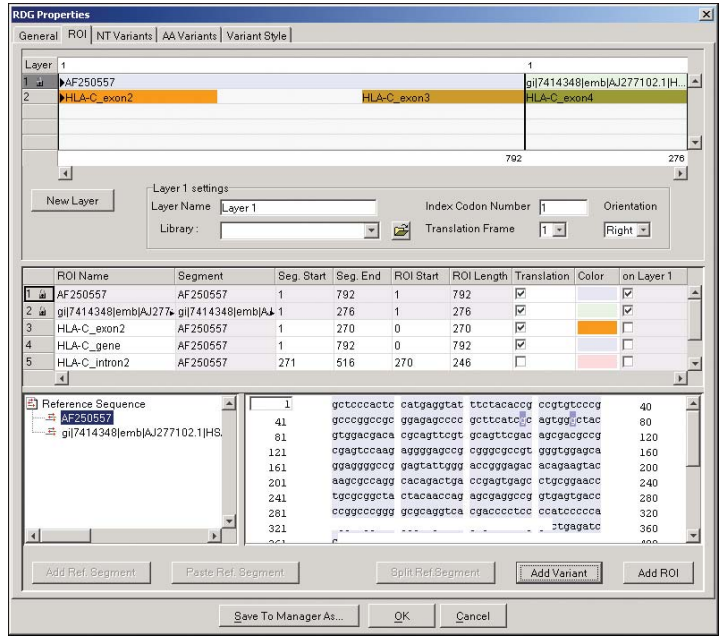


5. Select a style (color) with which you want the variant to be displayed, then enter a description of the variant, if desired.
6. Click **Create Another** to add more variants, or click **OK** to save the variant to the RDG.

Adding a Variant in the Project

To add a variant in the project:

1. From the Project window, select **Analysis > RDG Properties**.
2. Select the **ROI** tab.
3. Indicate your variant by doing one of the following:
   - Select the base that corresponds to the substitution variant or range of bases for a deletion variant.
   - Click the position at which you want an insertion variant.
4. Select **Add Variant**.



5. In the New NT Variant dialog box, note that the Position and the Reference base are already entered.
6. Select the type of variant by clicking **Base Change**, **Insertion**, or **Deletion,** then enter the Variant base.
7. Select a style for the variant, then enter a description of the variant, if desired.
8. Click **OK** to save the variant to the project.

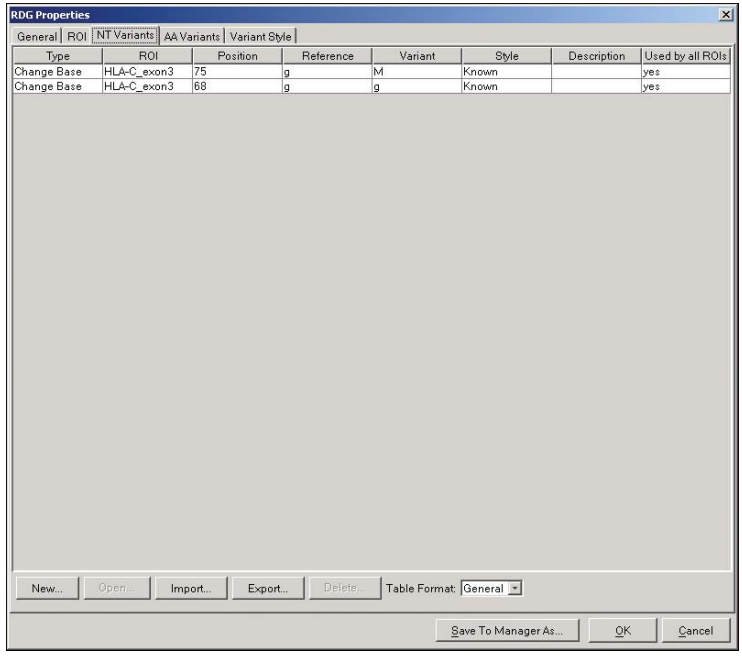
Importing Variants to the Project

When you import variants into a project, they must be in one of the following configurations:

- Tab-delimited text file format
- Text file format containing aligned sequences

To import variants into a project:

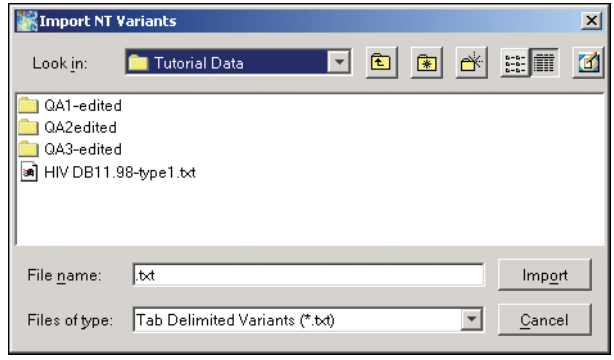
1. Open the RDG Properties dialog box and select the **NT Variants** tab.
2. Click **Import**.



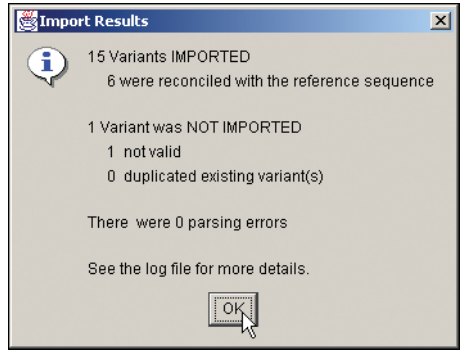
3. Browse to the appropriate file and select it.

   Note: The files must be tab-delimited text files as indicated in the File Types field.

4. Click **Import**.



5. Select the reference segment, then click **OK**.
6. After the data is imported, the Imports Results dialog box opens with information regarding the import.
7. Note the information, then click **OK**.



The variants now appear in the NT Variants tab of the RDG Properties dialog box.

Importing and Exporting Project Information

About Importing and Exporting

The purpose of importing and exporting project information is to transfer the project information to another computer.

You can export or import projects, project templates, reference data groups, nucleotide and amino acid variant tables, libraries, and analysis defaults from the SeqScape Manager. This allows you to examine and compare results from different Data Stores.

Note: The export and import functions of SeqScape Manager use the file extension CTF.

Importing from SeqScape Manager

To import from SeqScape Manager:

1. Select **Tools** > **SeqScape Manager**.
2. Select any tab into which you want to import.
3. Click **Import**.
4. Navigate to the file that you want to import.
5. Click **Import**.

   The imported file appears in the list under the appropriate tab.

Exporting from SeqScape Manager

To export from SeqScape Manager:

1. Select **Tools** > **SeqScape Manager**.
2. Select any one of the tabs from which you want to export.
3. Select the file that you want to export from the list, then click **Export**.
4. Navigate to a location to export.
5. Rename the file, if necessary, using the .ctf extension.
6. Click **Export**.

   The exported file is available to import into another project.

Viewing the Results 7

This chapter contains:

Workflow for This Chapter

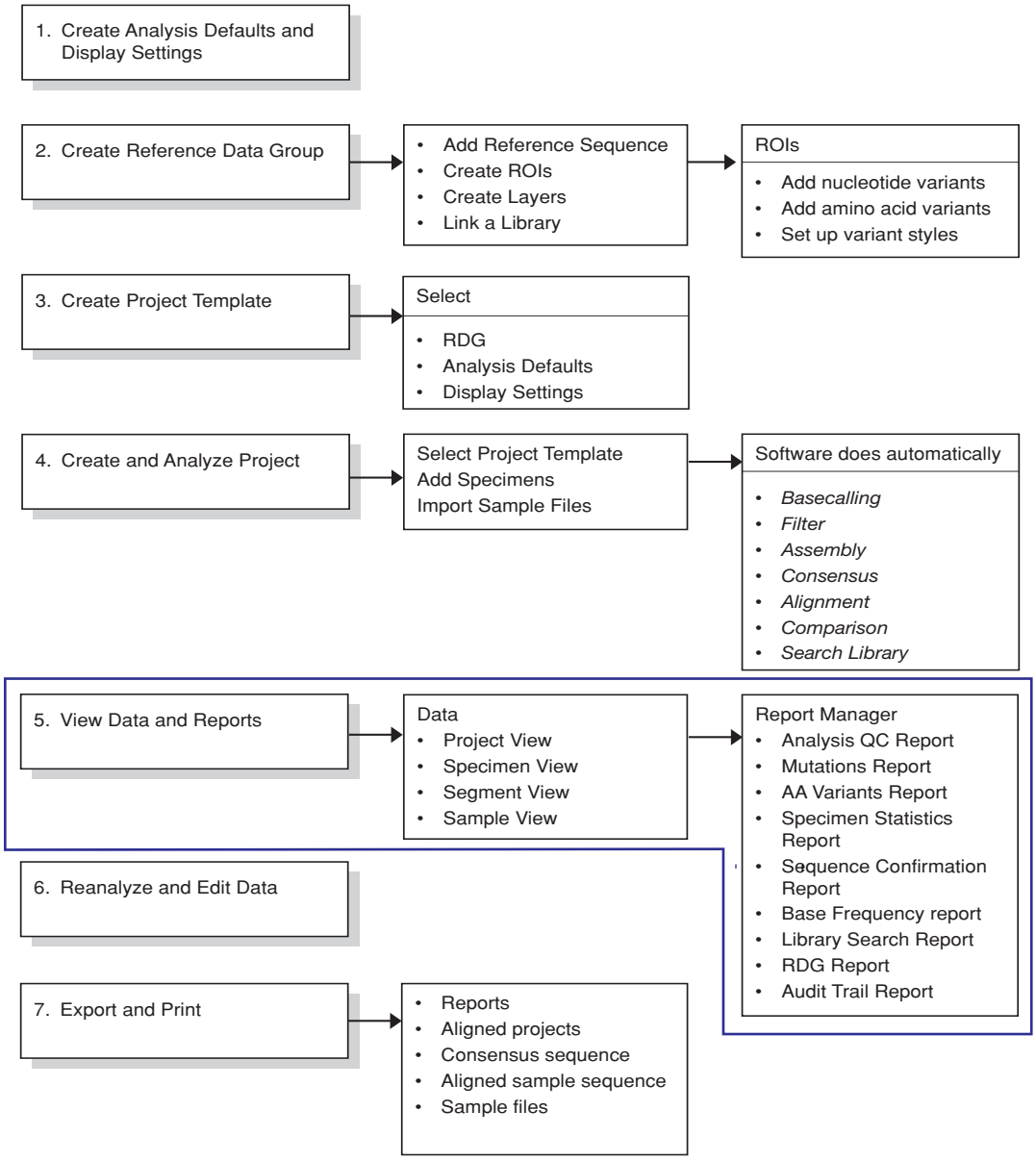


Figure 7-1 Showing the View Data and Reports Steps

About the Project Data

View Formats

You can view the results in multiple formats:

- Project view – A summary of all the specimen consensus sequences
- Specimen view – A summary of all the segment sequences within each specimen
- Segment view – A summary of all the sample sequences within each segment
- Sample view – A summary of the data for each sample

Data Display Conventions

The sequence data is displayed using the following conventions:

- Every mixed base (or choice of mixed bases) is represented as a single IUB code. For more information, see Appendix C, "Translation Tables."
- Spaces in aligned sequences are displayed as dashes and are not part of the original sequence.
- In the Dots view and in the collapsed NT view, characters that are identical to the reference are displayed as dots.
- The aligned reference sequence appears at the top of the table and the aligned sequences appear in the rows below in the Project view.

Quality Value Display

The QV (quality value) is displayed as a bar above each called base for the sample sequence and consensus sequence. The height of a bar corresponds to a 1–50 value that is determined by the analysis.

Note: For more information on quality values, see Chapter 10, "Sample and Consensus Quality Values."

Exporting and Printing Project Data

To export the project data, see "Exporting Data Files" on page 9-3, and to print data, see "Printing Data and Reports" on page 9-11.

Project Views

There are three project views, only one of which can be displayed at a time:

- Expanded NT
- Collapsed NT
- Expanded AA

Displaying the Project Views

To display project views:

1. Open the project of interest.
2. Select a layer in the Active Layer drop-down list.
3. At the top of the navigation pane, select the project icon.
4. Use the instructions in Table 7-1 to display the project views of interest.

Table 7-1 Project Views

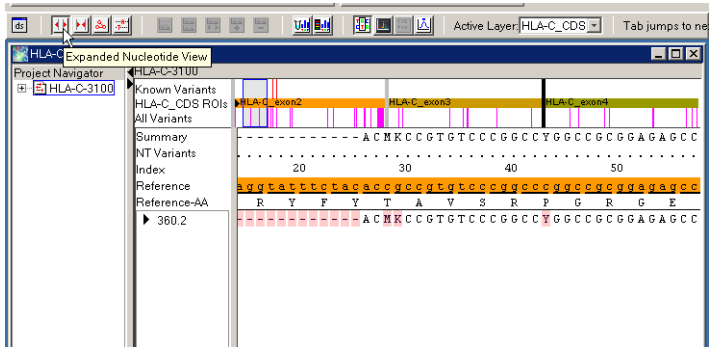
View	Procedure	Display
Expanded Nucleotide	Click  .	

Table 7-1 Project Views *(continued)*

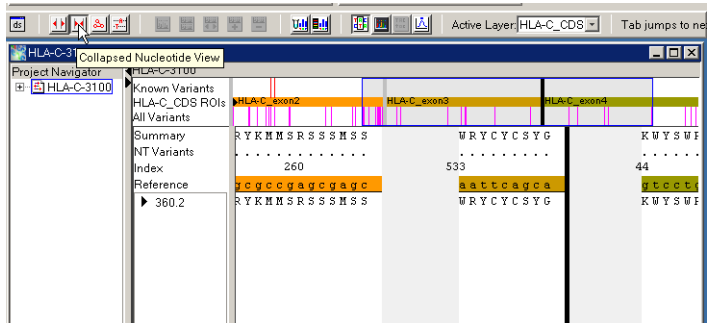
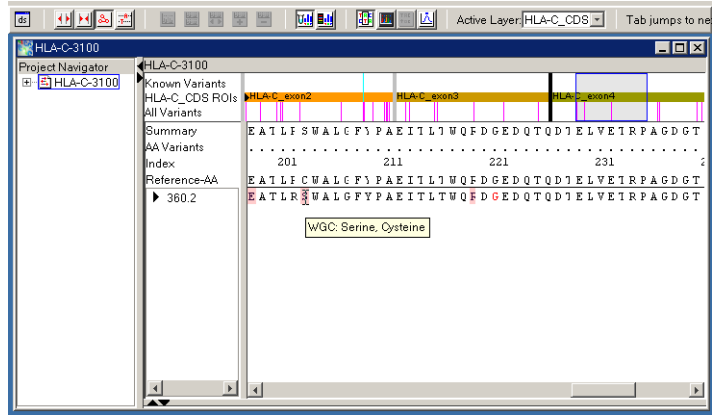
View	Procedure	Display
Collapsed Nucleotide	Click  .	 This view shows only those columns that differ from the aligned reference. Bases that match the reference sequence are displayed as dots, regardless of the state of the Dots setting. **Note:** Click  (Expanded Nucleotide View) to return to the expanded view.
Expanded Amino Acids (translation of the nucleotide sequence)	Click  .	 **Note:** Holding the pointer over an amino acid displays the possible translations and the codon at that position. **Note:** Bold red characters (default) indicate the location of a degenerate codon.

Table 7-1 Project Views *(continued)*

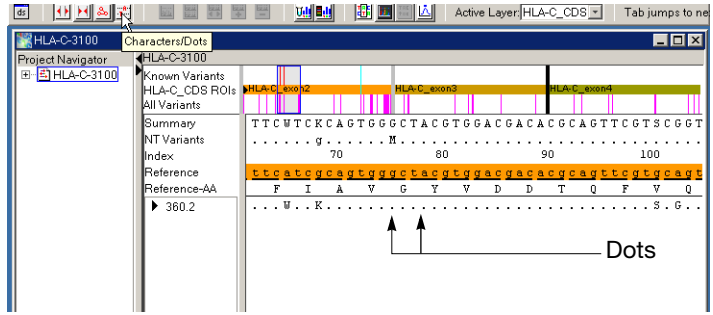
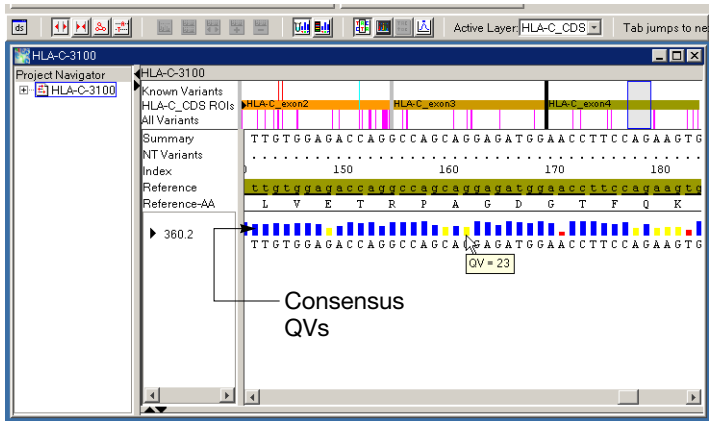
View	Procedure	Display
Dots	Click  .	 **Note:** The characters that are identical to the reference are displayed as dots.
Consensus QV	1. Click  . 2. Click  .	 **Note:** Holding the pointer over a base displays the numerical QV assignment for that base.

Table 7-1 Project Views *(continued)*

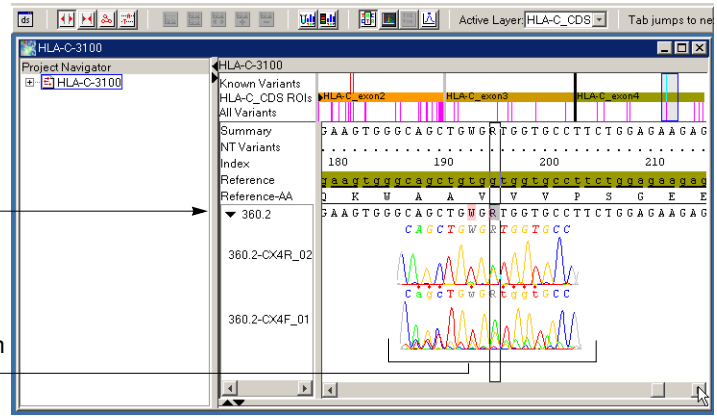
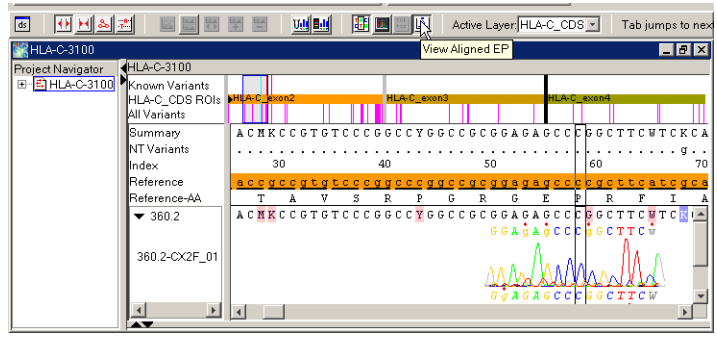
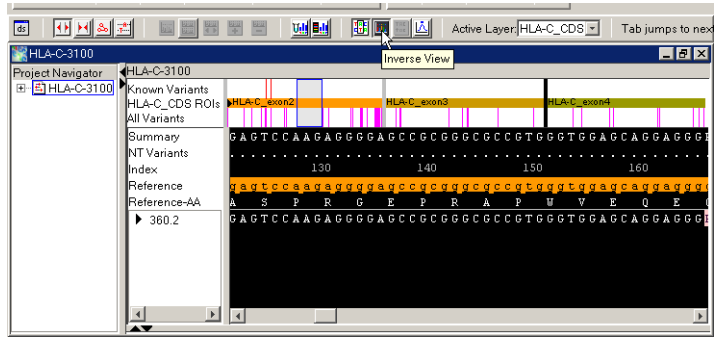
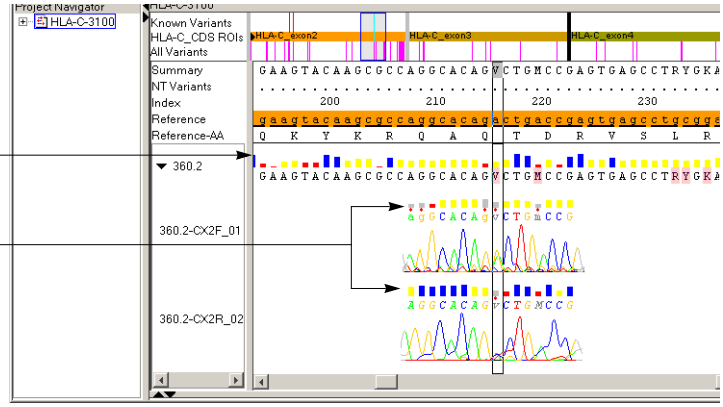
View	Procedure	Display
Electro-pherogram Snippet	1. In the Expanded Nucleotide or Dot view, select a base in the summary or specimen sequence. 2. Click the triangle next to the specimen name. Triangle Electropherogram snippets **Note:** Pressing Ctrl+Z centers any electropherogram snippets in the middle of the view.	
View Aligned EP	Click  .	

Table 7-1 Project Views *(continued)*

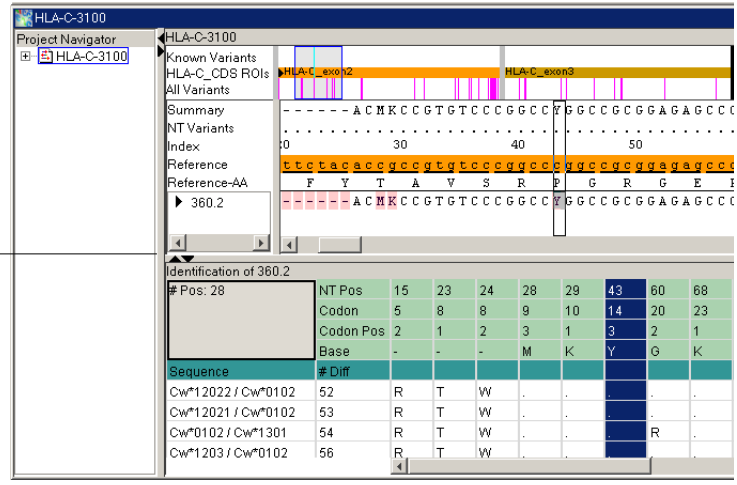
View	Procedure	Display
Inverse Video	Click  .	
QVs with Snippets or Dots	From the Snippet or Dots view, click  for sample QVs and/or click  for consensus QVs.	

Note: A gray QV bar indicates that the base has been edited and that the QV no longer holds true (until you restore the previous base).

Note: Holding the pointer over a base displays the numerical QV assignment for that base.

Table 7-1 Project Views *(continued)*

View	Procedure	Display
Identification	1. Drag the split bar at the bottom of the window up until you reach the desired height. 2. Select a base in a specimen sequence.	

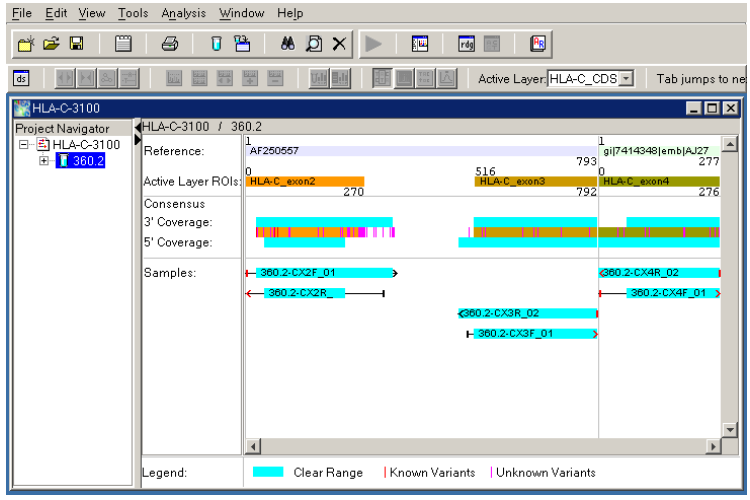


Specimen Views

The specimen result is displayed as a schematic of the location and orientation of all samples within a specimen with respect to the reference, ROIs in the current layers, and consensus sequence.

To display the specimen view:

1. Open the project of interest.
2. Select a layer in the Active Layer drop-down list.
3. In the navigation pane, open the project (if necessary), then select a specimen icon.



Segment Views

There are two segment views:

- Layout view – Displays a schematic of the location and orientation of the samples with respect to the consensus segment and the reference
- Assembly view – Displays the nucleotide sequence of the consensus and samples, sample electropherogram data, and view position.

Note: The view position in the Assembly view (blue box) is represented by red lines in the Layout view. Click the Layout view to navigate to a desired position in the Assembly view.

Table 7-2 describes the multiple Assembly view types.

Displaying the Segment Views

To display segment views:

1. Open the project of interest.
2. Select a layer in the Active Layer drop-down list.
3. In the navigation pane, open a specimen, then select a segment.
4. Use the procedures in Table 7-2 to display the segment views of interest.

Table 7-2 Segment Views

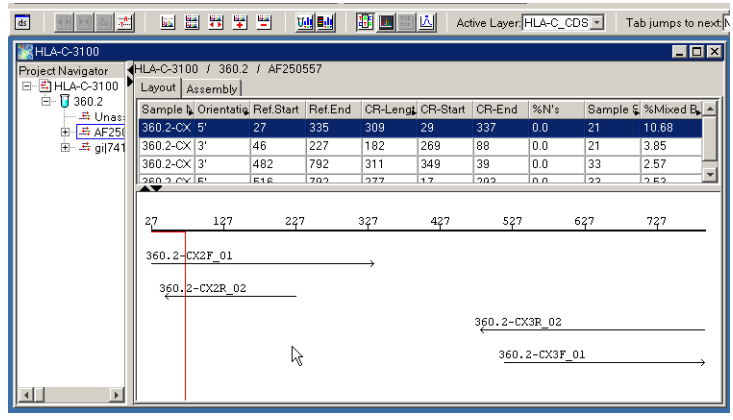
View	Procedure	Display
Layout	Select the **Layout** tab.	

Table 7-2 Segment Views *(continued)*

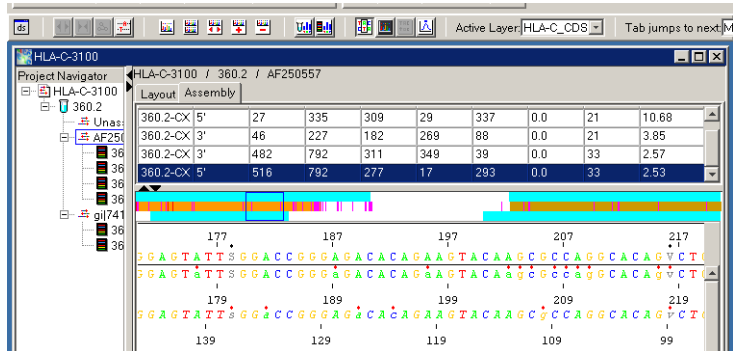
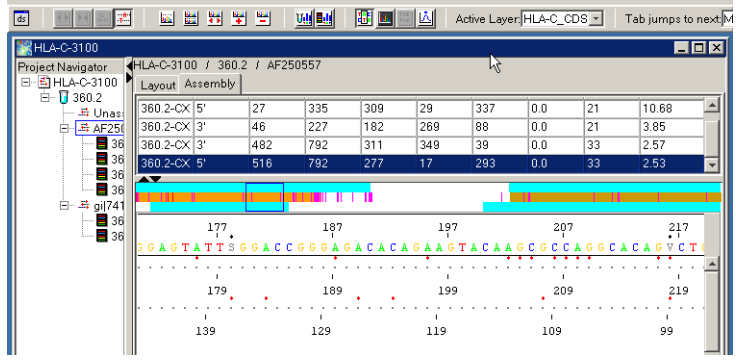
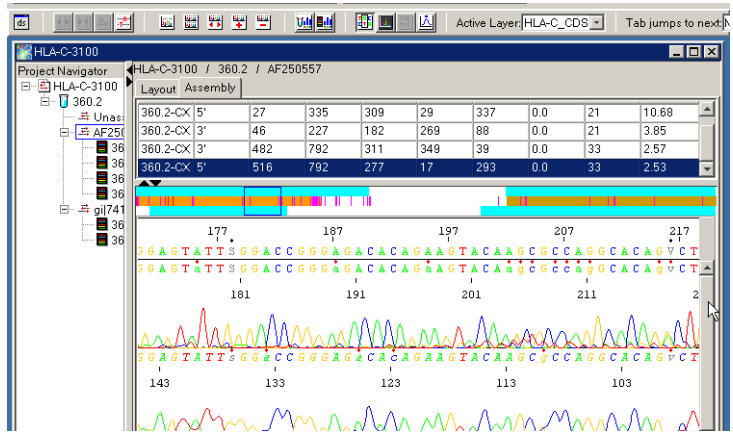
View	Procedure	Display
Sequence Assembly	Select the **Assembly** tab.	
Dot Assembly	1. Select the **Assembly** tab. 2. Click 	
Electro-pherogram Assembly	1. Select the **Assembly** tab. 2. Select a sample in the sample table. 3. Click  for multiple EPS or click  for one EP.	

Table 7-2 Segment Views *(continued)*

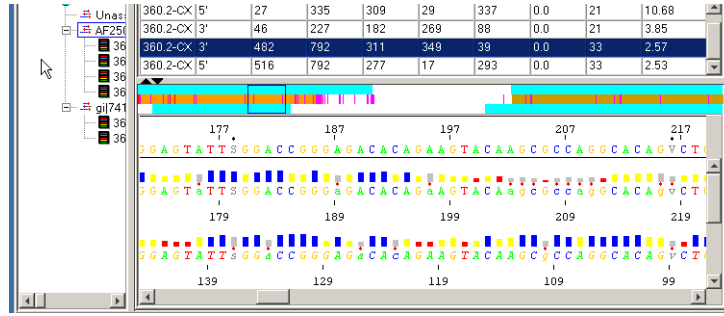
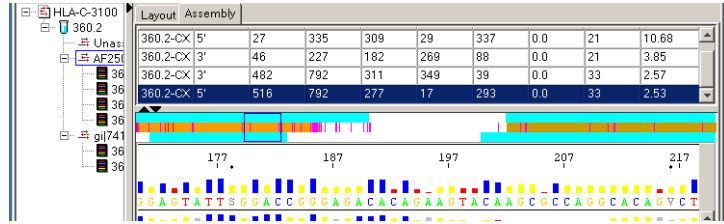
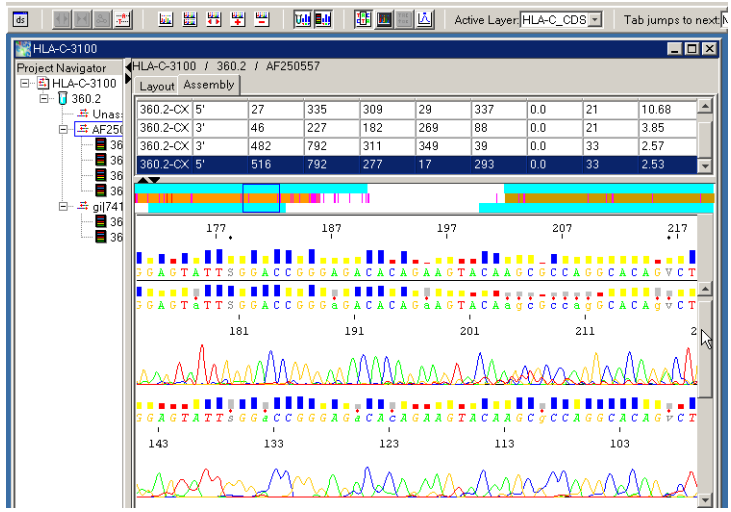
View	Procedure	Display
QV Assembly	1. Select the **Assembly** tab. 2. Click  for sample QVs and/or click  for consensus QVs.	 

Table 7-2 Segment Views *(continued)*

View	Procedure	Display
Assembly (The EP and QV views can be used with the Sequence or Dot view.)	1. Select the **Assembly** tab. 2. Click  . 3. Click  . 4. Click  .	 **Note:** A black dot above a base in the consensus sequence indicates a discrepancy between the consensus and the reference sequences, and a red dot above a sample base indicates the base was edited by the consensus caller. Options: • Use  (zoom tools) to zoom in/out the horizontal view. • Use  (Aligned EP) to scale horizontal EP peaks to be of even width and aligned to base calls. • Use  (View Column Selector) to turn on column selector, indicating position where base call is aligned to EP peak of original (not scaled) EP. • Use  (Inverse Video) to switch between white and black background displays. • Use Find and Find Again to search for text.

Sample Views

The sample result includes all the data characteristics of a sample. Sample data characteristics are displayed in the following tabs:

Table 7-3 Sample View Tabs

Tab	Displayed Information
Annotation	Information about the data and its analysis.
Sequence	Sequence of the sample in NT codes. For readability, the display clusters the sequences into substrings of 10 characters each, separated by blanks.
Features	Calculated clear range and multiple base positions.
Electropherogram	Electropherogram and basecall data for the sample. The data excluded from the clear range is shown in gray.
Raw	Raw data collected by the genetic analyzer.

Displaying the Sample Views

To display sample views:

1. Open the project of interest.
2. In the navigation pane, open a specimen, then open a segment.
3. Select a sample, then select a tab (see Table 7-3).
4. Select a new tab to change the view.
5. To view a different sample, select a new sample from an open segment, then select a tab.

Examples of the five tab views are displayed in Table 7-4, “Sample Views,” on page 7-16.

Table 7-4 Sample Views

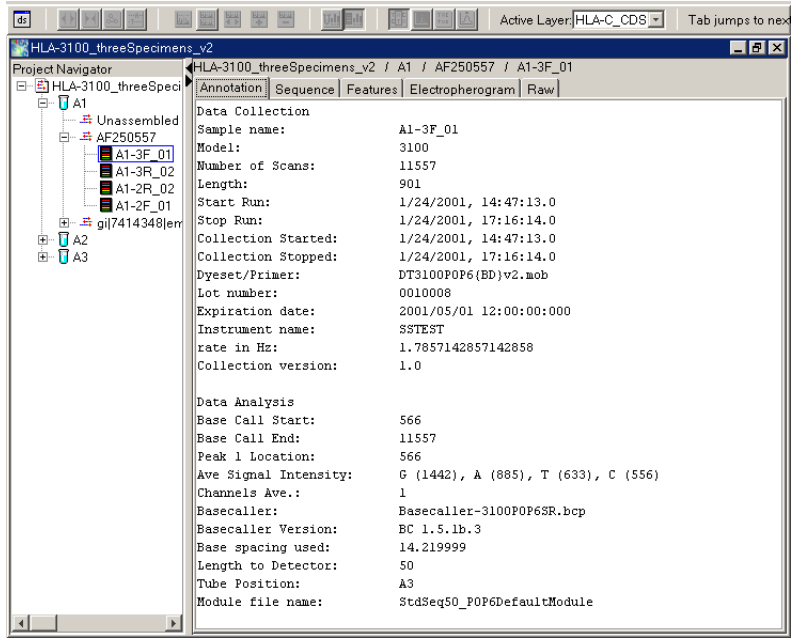
Tab	Display
Annotation	

Table 7-4 Sample Views *(continued)*

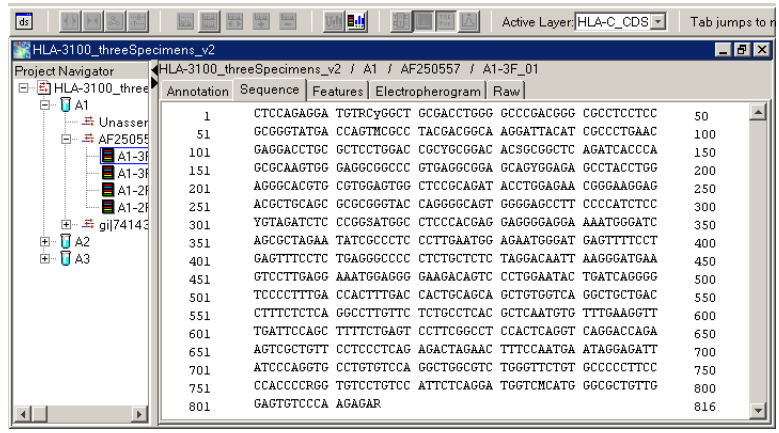
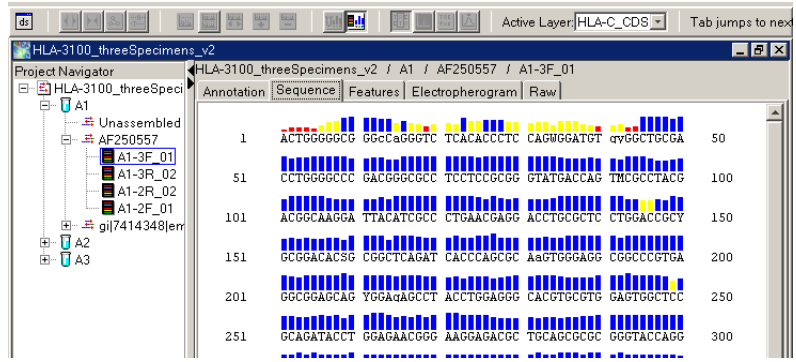
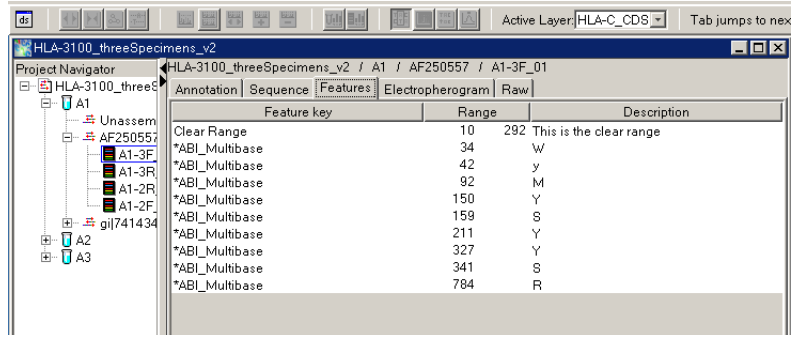
Tab	Display
Sequence	 Use  (Show/Hide Sample QV) to display/hide sample QVs. 
Features	

Table 7-4 Sample Views *(continued)*

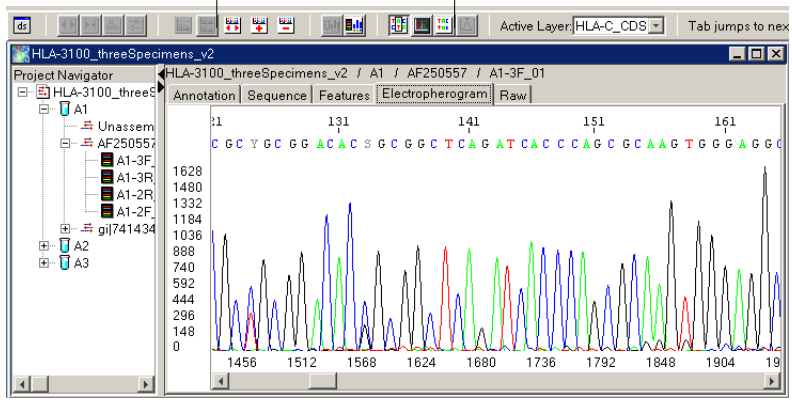
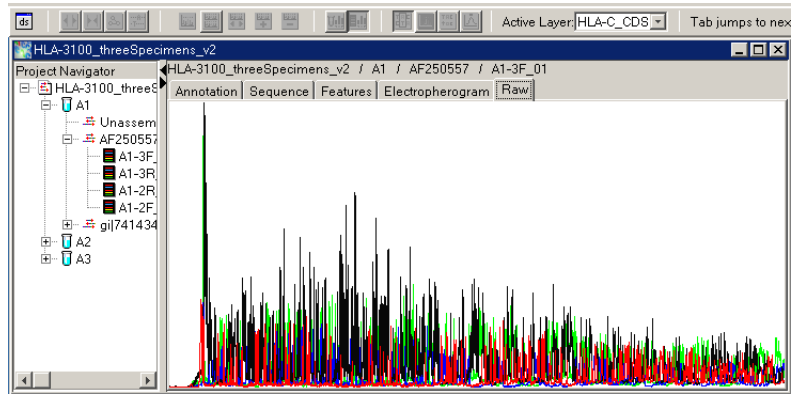
Tab	Display
Electropherogram	Active tools  Options: • Use (zoom tools) to zoom in/out the horizontal view. • Use (Show/Hide Sample QV) to display/hide sample QVs. • Use (Inverse Video) to switch between white and black background displays. • Use (View Original Sequence) to display/hide the original sequence called by the software. • Use Find and Find Again to search for text.

Table 7-4 Sample Views *(continued)*

Tab	Display
Raw	

Viewing Variants

Two methods to view variant data are presented here.

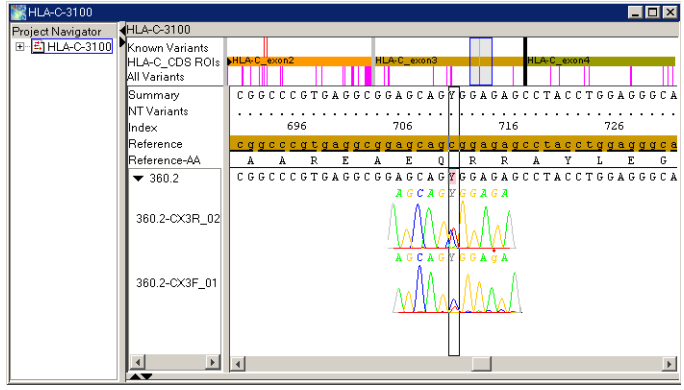
Note: To edit variant data, see “Editing Variants” on page 8-18.

To view variant data:

Method 1

1. Open the project of interest.
2. Click a consensus base.
3. View the electropherogram snippets by clicking the triangle that appears next to the specimen name.
4. In the Tab Jump to Next drop-down list, select **Multiple**, then select **Known Variant** and **Unknown Variant**.
5. Press **Tab** to move to the next variant or press **Shift-Tab** to move to the previous variant.

   Note: Pressing Ctrl+Z moves any electropherogram snippets of the selected variants to the middle of the view.



Method 2

1. Open the project of interest.
2. Select **Analysis > Report Manager** or click  .
3. In the navigation pane, select the report you want to view.
4. Select **Window > Tile**.
5. Review the positions by selecting a base change in the Mutation table. This action brings the alignment view to the correct position in the alignment.

Saving Your Data

When you finish, select **File > Save Project** or click  .

IMPORTANT! Any changes you make are saved and overwrite the existing project.

About the Reports

After the data is analyzed, you can view, export, and print reports. Reports can help you troubleshoot your results because reports contain hyperlinks to the primary sequence data. You can use reports with project results to evaluate your samples, modify the analysis settings, and edit the basecalling.

Types of Reports

Nine report types are generated with every project analysis. All reports are contained in one Report Manager window. Each project has its own Report Manager window containing the following reports:

- Analysis QC
- Mutations
- AA Variants
- Specimen Statistics
- Sequence Confirmation
- Base Frequency
- Library Search
- RDG
- Audit Trail

Note: Only one report can be viewed at a time.

Common to all reports is a Summary table that includes project information and the specimens in the report.

Exporting and Printing Reports

To export a report, see “Exporting Reports” on page 9-8, and to print a report, see “Printing Data and Reports” on page 9-11.

Analysis QC Report

The report contains four separate tables. All blue text is hyperlinked to the project navigator.

Table 7-5 Parts of the Analysis QC Report

Table	Description
Summary	Displays project information and the specimens in the report.
Specimen Analysis	Displays specimen analysis results, specimen score (average consensus QV) and total number of variants.
Sample Analysis	Displays sample analysis errors and details.
Possible Heterozygous Indel Mutations	Displays possible mutations and their positions and size for each specimen.

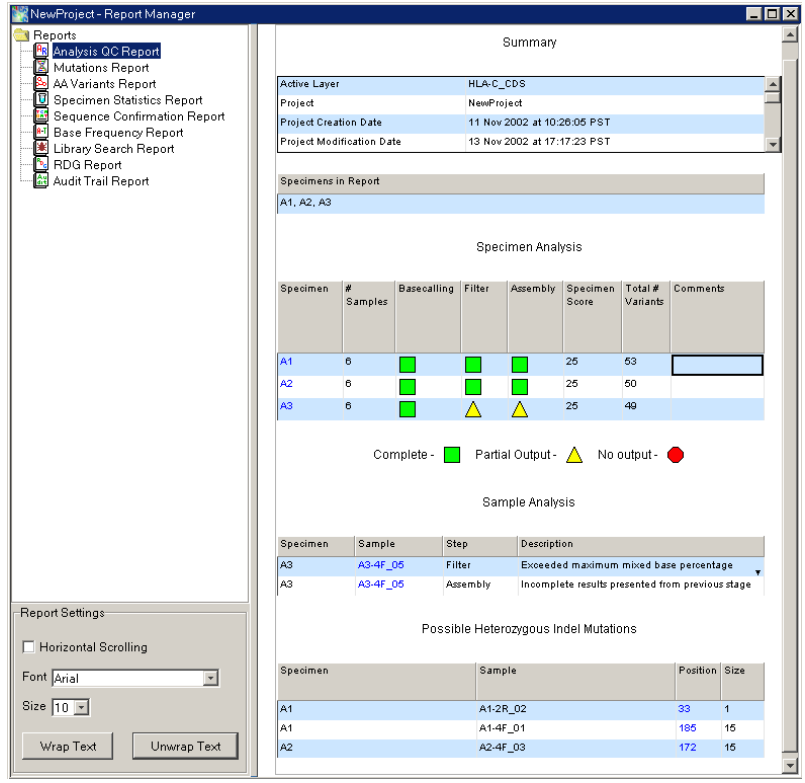


Figure 7-2 Analysis QC Report

Mutations Report

The report contains two separate tables. All blue text is hyperlinked to the project navigator.

Table 7-6 Parts of the Mutation Report

Table	Description
Summary	Displays project information and the specimens in the report.
Mutation	Displays the bases changed, ROI, position, length, type, QV, and effect information for each mutation detected in a specimen.

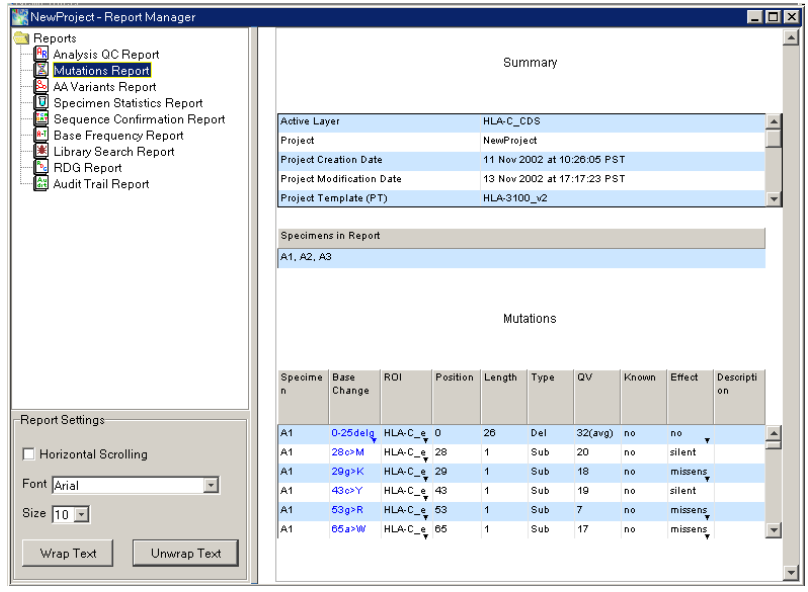


Figure 7-3 Mutations Report

The Mutations report includes a column that provides a predicted "effect" for each nucleotide variant. Table 7-7 on page 7-25 describes the possible values in the Effect column.

Table 7-7 Predicted Effects of Nucleotide Variants

Effect	Description
Missense	The substitution variant codes for an amino acid substitution.
Nonsense	The substitution variant codes for a terminator codon. (In a mixed codon, if any codon is a terminator codon “nonsense” will be displayed).
Silent	The substitution variant is in a coding region but does not code for an amino acid change.
Frameshift Insertion	The insertion variant is in a coding region and codes for a frameshift in translation (the size of the insertion is not a multiple of three).
Frameshift deletion	The deletion variant is in a coding region and codes for a frameshift in translation (the size of the deletion is not a multiple of three).
In-frame insertion	The insertion variant is in a coding region and does not code for a frameshift in translation (the size of the insertion is a multiple of three).
In-frame deletion	The deletion variant is in a coding region and does not code for a frameshift in translation (the size of the deletion is a multiple of three).
Non-coding	The variant is not in a coding region.
Partial codon	The variant is in a coding region, but occurs at the beginning or end of the sequence, where you do not know the full three-base codon sequence.
No information	The variant is a result of the consensus sequence not completely covering the reference sequence. These are not real variants, so you cannot predict a real effect.

AA Variants Report

The report contains two separate tables. All blue text is hyperlinked to the project navigator.

Table 7-8 Parts of the AA Variant Report

Table	Description
Summary	Displays project information and the specimens in the report.
AA Variant	Displays the AA changed, position, length, type, and description for each variant detected in a specimen.

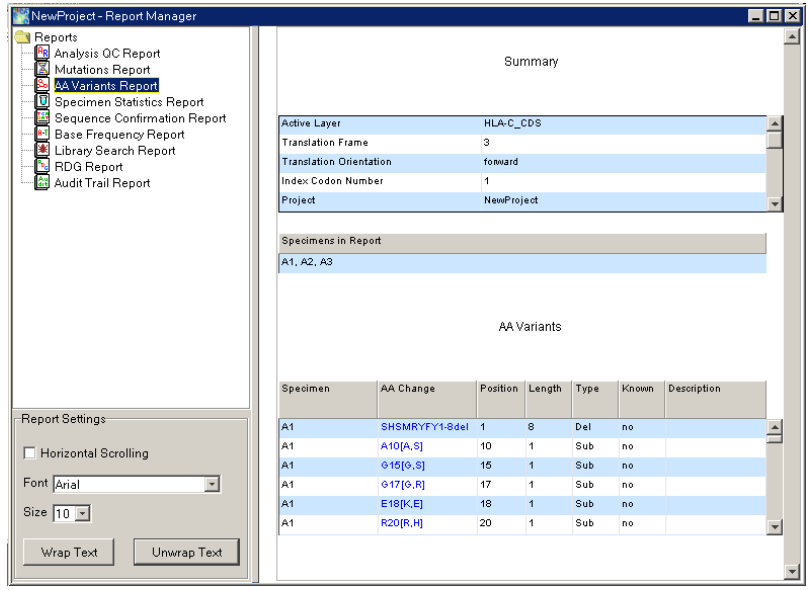


Figure 7-4 AA Variant Report

Specimen Statistics

The report contains three separate tables.

Table 7-9 Parts of the Specimen Statistics Report

Table	Description
Summary	Displays project information and the specimens in the report.
Specimen Statistics	Displays the bases changed, ROI, position, length, type, QV, and effect information for each mutation detected for each specimen.
Sample Results	Displays the specimen, segment, assembly status, calculated clear range, sample score (average QV) and % mixed bases for each sample.

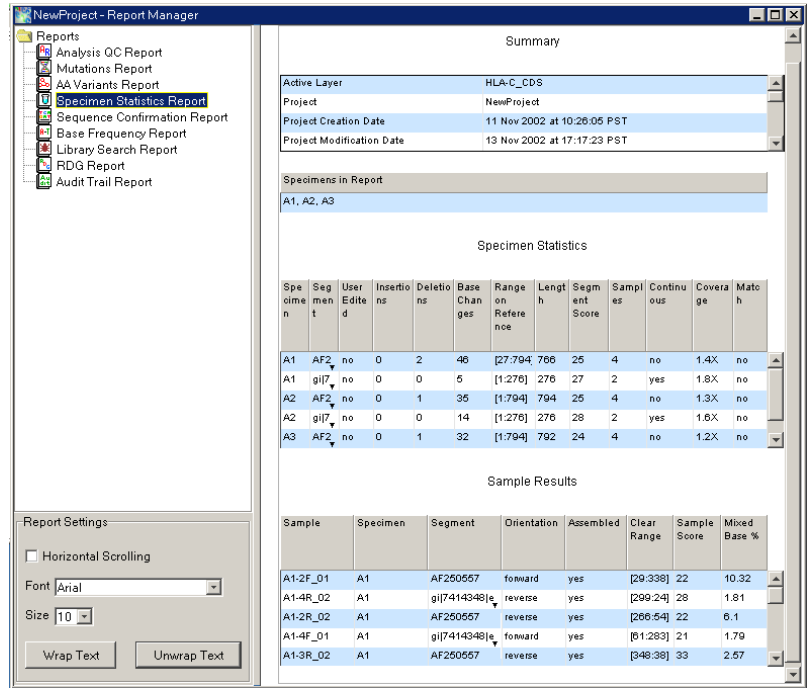


Figure 7-5 Specimen Statistics Report

Sequence Confirmation Report

The report contains two separate tables.

Table 7-10 Parts of the Sequence Confirmation Report

Table	Description
Summary	Displays project information and the specimens in the report.
Sequence Confirmation	Displays the match, the number of insertions, deletions and bases, and the amount of coverage and whether it is continuous for each specimen.

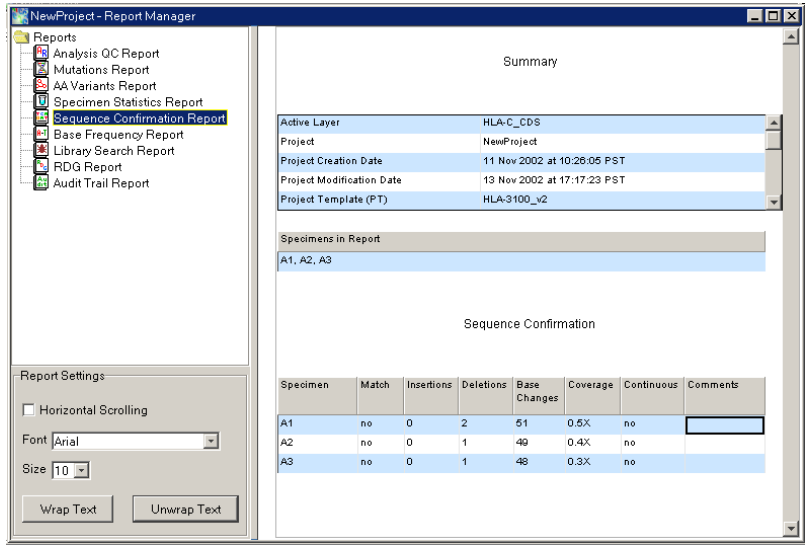


Figure 7-6 Sequence Confirmation Report

Base Frequency Report

The report contains two separate tables.

Table 7-11 Parts of the Base Frequency Report

Table	Description
Summary	Displays project information and the specimens in the report.
Base Frequency	Displays the reference, ROI, and the % of each base and space for each variant position.

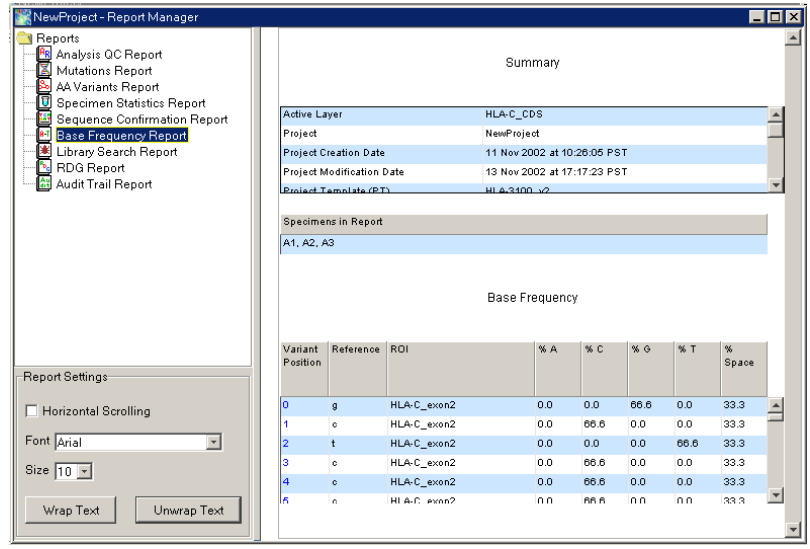


Figure 7-7 Base Frequency Report

Library Search Report

The report contains five separate tables (see Figure 7-8 on page 7-31).

Table 7-12 Parts of the Library Search Report

Table	Description
Summary	Displays project information and the specimens in the report.
Library	Displays the library and information used in the search.
Specimen Results	Displays the match status, crucial position and constant position errors for each specimen.
Hit List	Displays the library matches found, their scores (closest match), and mismatch information for each specimen.
Constant Positions Errors	Displays the position, specimen base and library base for each specimen and ROI. The values in the Position column are hyperlinked to the project navigator.

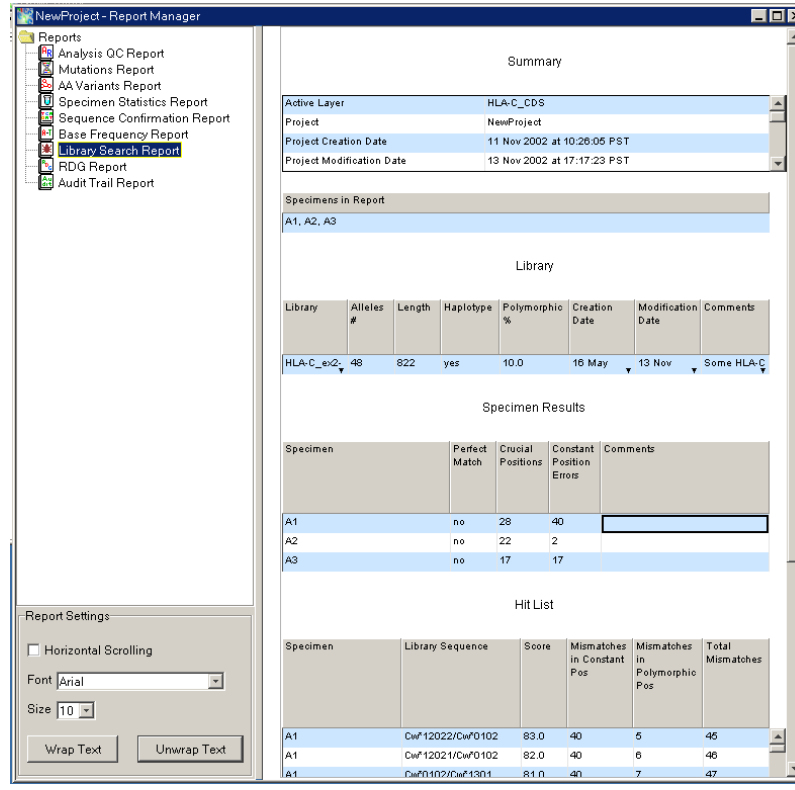


Figure 7-8 Library Search Report

RDG Report

The report contains three separate tables.

Table 7-13 Parts of the RDG Report

Table	Description
Summary	Displays project information and the specimens in the report.
Layers	Displays a summary of the information for each layer in the project as defined in the RDG.
ROIs	Displays a summary of the information for each ROI as defined in the RDG.

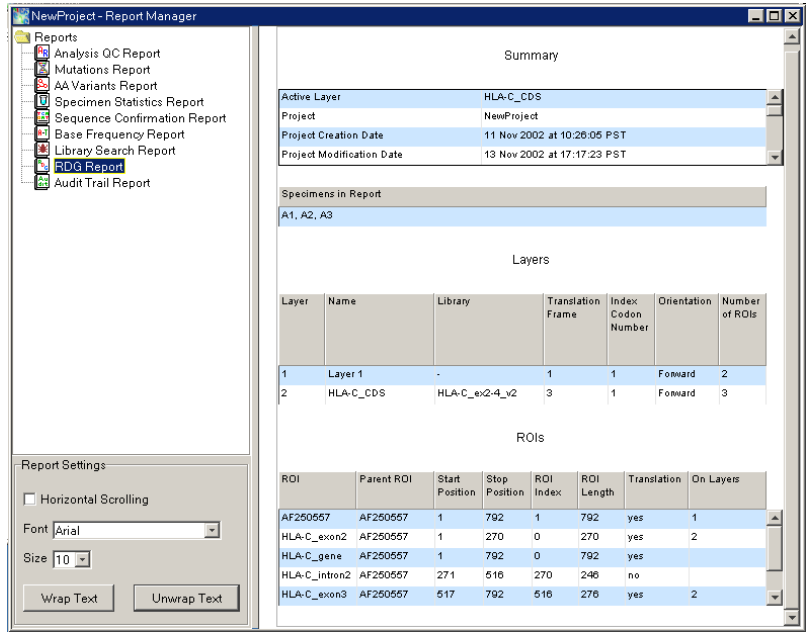


Figure 7-9 RDG Report

Audit Trail Report

The report contains two separate tables.

Table 7-14 Parts of the Audit Trail Report

Table	Description
Summary	Displays project information and the specimens and samples in the report.
Audit Trail	Displays a record of the edits and changes made to data in a project, if the audit trail feature is on.

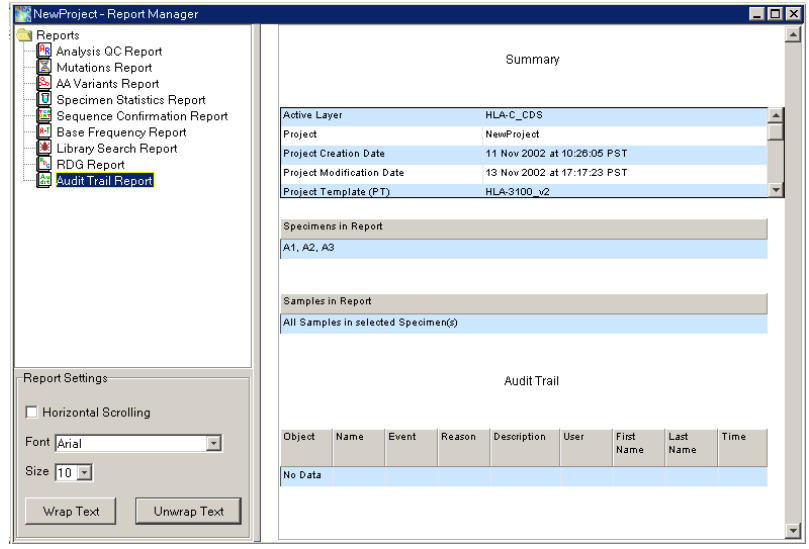


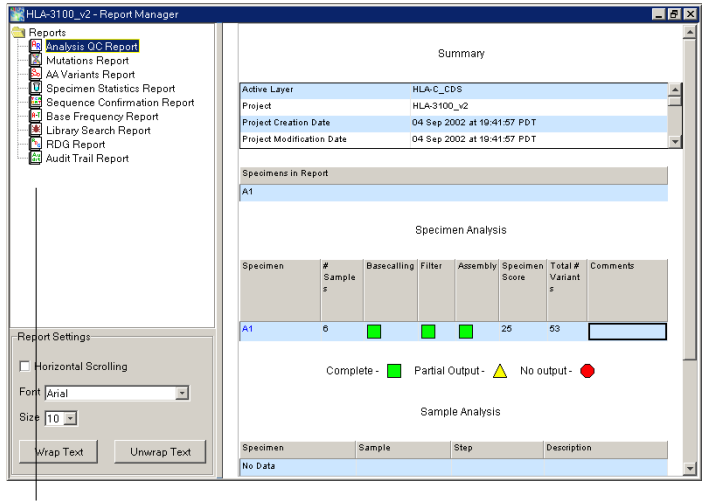
Figure 7-10 Audit Trail Report

Viewing the Reports

The data in the reports is filtered, based on the view (project, specimen, segment or sample) selected in the navigation pane of the project and the layer selected from the Active Layer drop-down menu.

To view a report:

1. Open the project of interest, then select the active layer.
2. In the navigation pane, select the project, specimen, segment, or sample view.
3. Select **Tools** > **Report Manager** or click  .



Select report type here

4. In the navigation pane, select the report you want to view.
5. To view other reports, select the new report in the navigation pane.

   Note: Only one report can be viewed at a time.

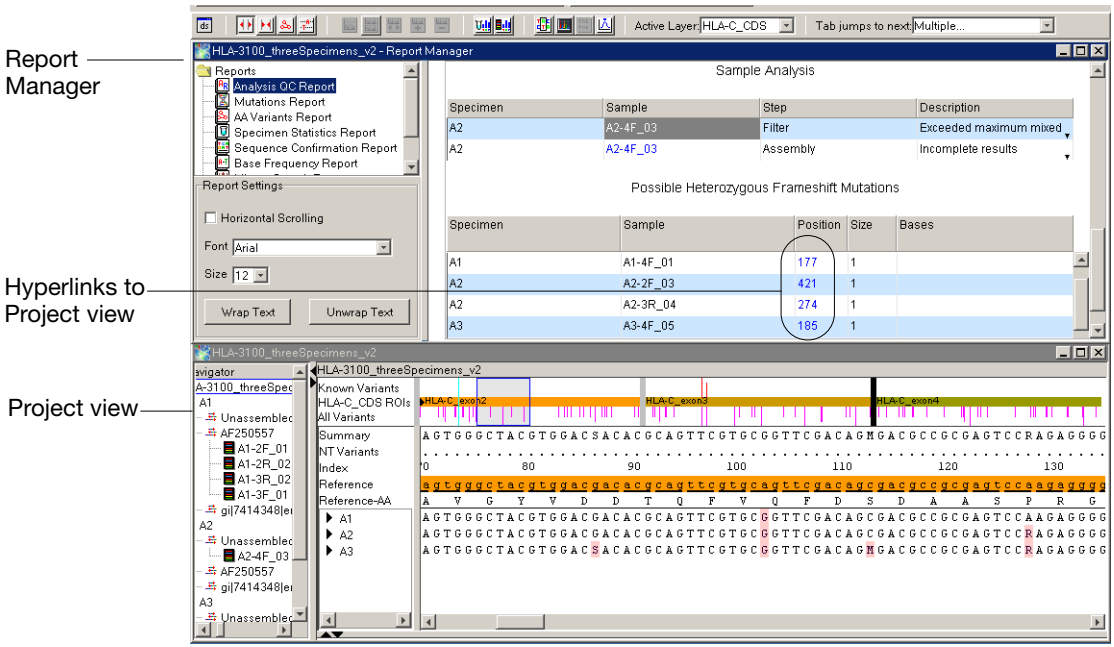
6. To update the reports with additional data:
   a. In the project, select additional or different specimen/samples.
   b. Click  to update the data in the open report.

Viewing the Reports and Project Results

When you view reports, you can tile the report with the Project window so that you can easily view the data when you use a hyperlink.

To view the project results and reports together:

1. Open the project of interest.
2. Select a layer in the Active Layer drop-down list.
3. Select **Analysis > Report Manager** or click  .
4. In the navigation pane, select the report you want to view.
5. Select **Window > Tile**.
6. Click a hyperlink (blue text) in the report, then view the data in the Project view.



Customizing the Reports

Customizing Text Settings

To customize the view, use the Report Settings section which is located in the bottom left corner of the Report Manager window.

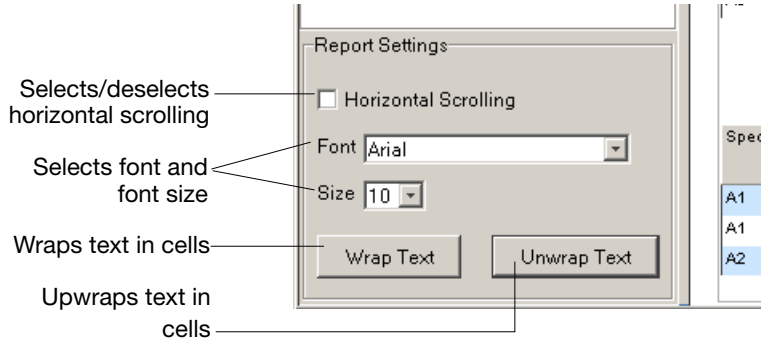


Figure 7-11 Options Available in the Report Settings

To customize the font and text in the cells:

1. Select or deselect **Horizontal Scrolling** check box. The default is off.
2. Select your font type and font size in the appropriate drop-down lists. The default is font and size is Arial 10.
3. Click **Wrap Text** or **Unwrap Text**. Examples of wrapped and unwrapped text are shown below.

Specimen	Sample	Step	Description
A3	A3-4F_05	Filter	Exceeded maximum mixed base
A3	A3-4F_05	Assembly	Incomplete results presented from

Unwrapped text

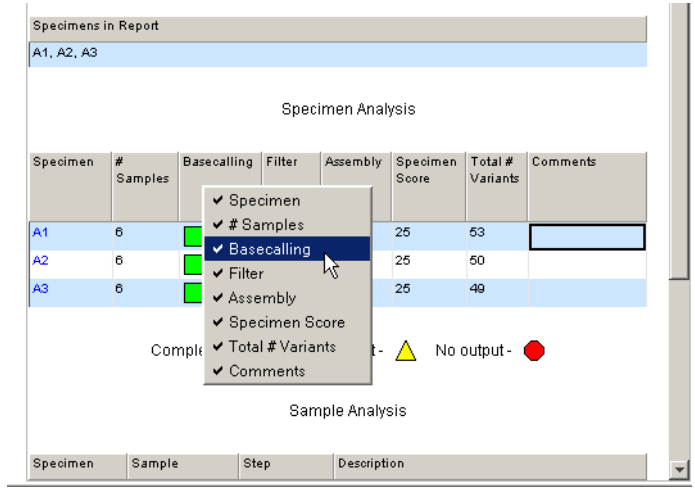
Specimen	Sample	Step	Description
A3	A3-4F_05	Filter	Exceeded maximum mixed base percentage (45.901638%>35.0%)
A3	A3-4F_05	Assembly	Incomplete results presented from previous stage

Wrapped text

Customizing the Data View

To customize the information displayed in the report:

1. Right-click any column heading of a table. A list of the column headings in the table is displayed.



2. To hide a column, deselect the column heading.
3. Repeat steps 1 and 2 to deselect additional headings.
4. To redisplay a column, right-click any column heading, then select the column heading.
5. To sort the data A to Z or Z to A in a Sample Details or Errors table column, double-click the column heading. Double-click again to sort in the opposite direction.
6. To customize the table header and footer information, see “Customizing Header and Footer Display” on page 9-11.

Reanalyzing and Editing Data 8

This chapter contains:

Workflow for This Chapter

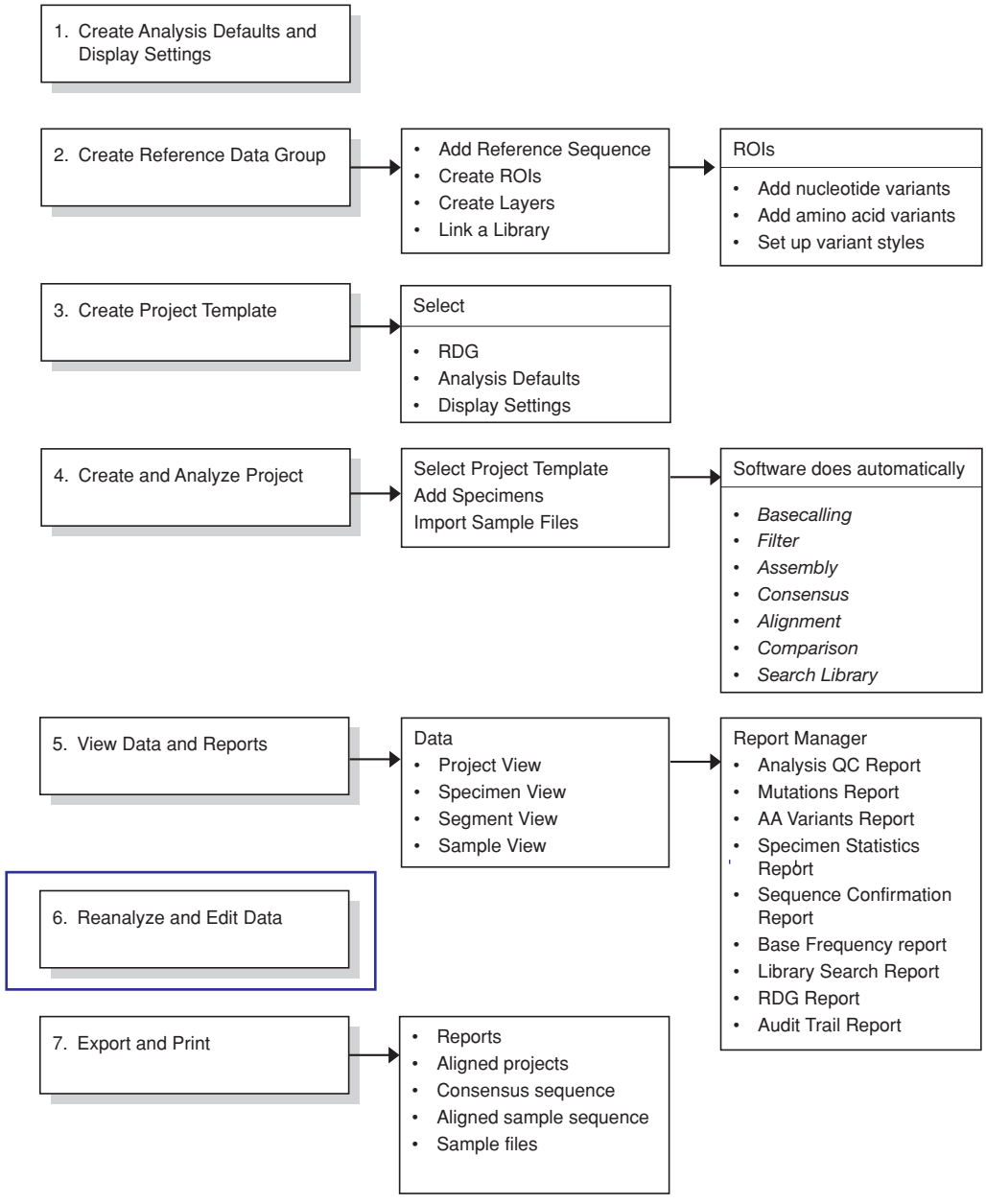


Figure 8-1 Reanalyzing and Editing Data Step

About Analysis Parameters

Introduction

The analysis parameters (basecaller and DyeSet/Primer file) associated with every sample file are used when the sample files are analyzed.

Sometimes poor project results can be corrected or improved by changing certain analysis settings and applying the new settings to the affected samples.

Common examples of errors that affect basecalling are:

- Incorrect stop point selected
- Bad base spacing
- Poor quality data
- Incorrect basecaller and/or dyeset/primer used for basecalling
- Wrong peak 1 location and start point calculated by the software

Note: Refer to the ABI PRISM® *DNA Sequencing Analysis Software User Guide* for instructions to define a new peak 1 and the start and stop point locations.

Viewing Analysis Parameters in the Sample Manager

You can use the Sample Manager to display sample files and their current analysis information including the basecaller and DyeSet/Primer files (see Figure 8-2 on page 8-4). The analysis parameters can be modified and applied to samples. You can apply these changes to one sample, some samples, or all samples in the Sample Manager.

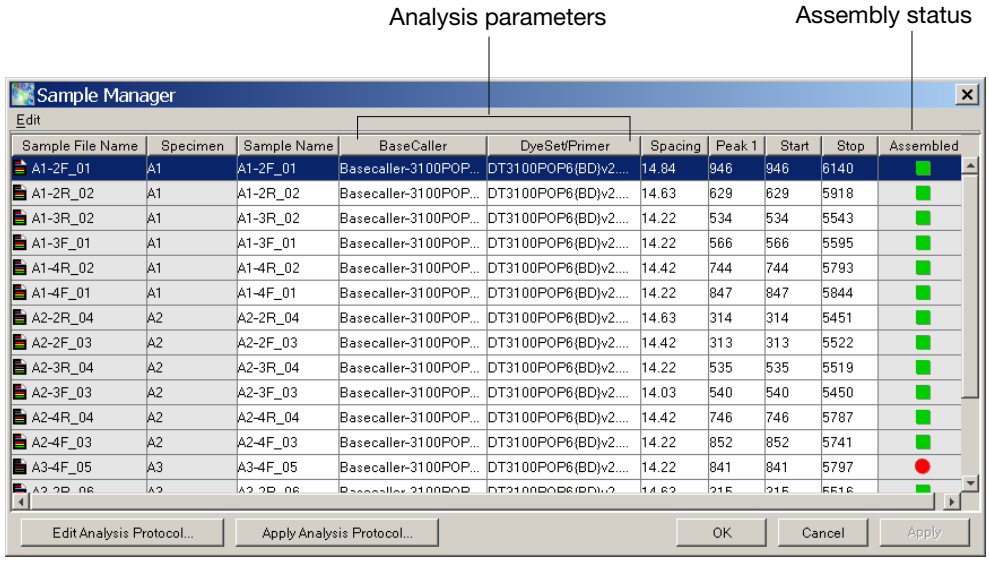


Figure 8-2 Sample Files in the Sample Manager

The Sample Manager window displays the following information:

Table 8-1 Information in the Sample Manager

Column Heading	Description
Sample File Name	Information from the plate record and project. It cannot be changed in the Sample Manager.
Specimen	Information from the plate record and project. It cannot be changed in the Sample Manager.
Sample Name	Name of the sample, taken from the plate record. It can be changed.
Basecaller	Algorithm used to call the bases. It can be changed.

Table 8-1 Information in the Sample Manager *(continued)*

Column Heading	Description
DyeSet/Primer	A DyeSet/Primer file corrects for mobility shifts and color-code changes, depending on which chemistry was used. DyeSet/Primer files are sometimes known as mobility or .mob files. All mobility files have the extension .mob. It can be changed.
Spacing	The number of scan points from the crest of one peak to the crest of the next peak. During basecalling, a spacing calibration curve is applied to the data to determine a base spacing value.
Peak 1	The first data point that is from the sample, not including primer peaks in dye primer chemistries. It is the reference point for the spacing and mobility corrections performed by the basecalling software.
Start	The raw data point where the basecalling starts in the sample file. The Start Point is normally the same as the beginning of the first base peak.
Stop	Specifies the last raw data point to be included in the basecalling. If the default Stop Point is used, this endpoint is the last data point in the file.
Assembled	Displays the assembly status of the sample. A green box means assembled and a red circle means not assembled.

Changing the Analysis Parameters in the Sample Manager

Adding Samples to the Sample Manager

To add samples to the Sample Manager:

1. Open the project of interest.
2. Select a layer in the Active Layer drop-down list.
3. In the navigation pane:

To add all …	Select the …
Samples in a project	Project icon
Samples in a specimen	Specimen
Selected samples in a segment	Segment
Selected sample(s)	Sample(s)*

* Use the Shift key to select contiguous samples, or use the Ctrl key to select noncontiguous samples.

4. Select **Analysis > Sample Manager**.

   The selected files are displayed in the Sample Manager.

Changing Basecaller and DyeSet/Primer Files

Note: Use the basecaller and DyeSet/Primer tables in Appendix B to select the correct combination of files.

To change the basecaller and/or DyeSet/Primer file:

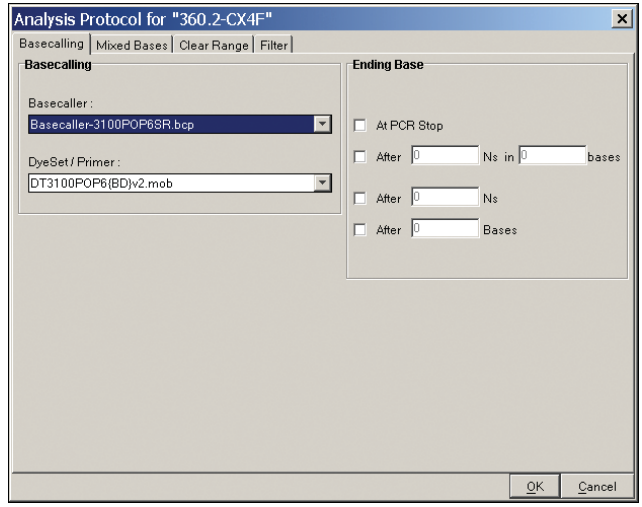
1. In the Sample Manager, select the sample you want to change.
2. In the Basecaller drop-down list, select a new basecaller.
3. In the DyeSet/Primer drop-down list, select a new DyeSet/Primer file.
4. To change multiple samples, use the Fill Down function.
5. Click **Apply**.
6. Click **OK**.
7. Click  .

Changing the Analysis Parameters in an Analysis Protocol

Editing an Analysis Protocol

To edit an analysis protocol:

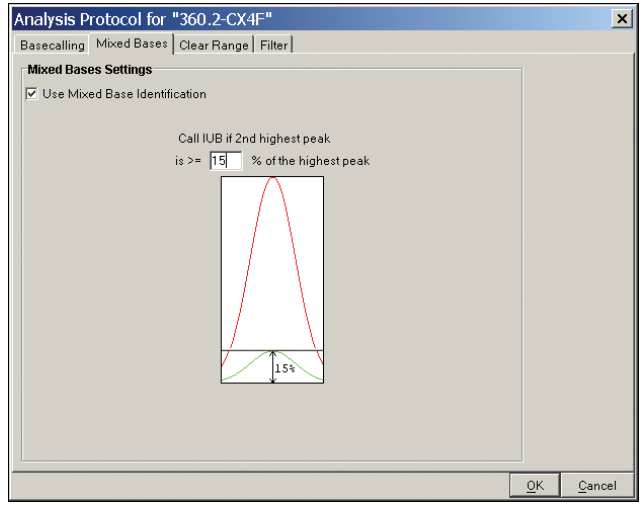
1. Add the samples to the Sample Manager (see “Adding Samples to the Sample Manager” on page 8-6).
2. In the Sample Manager window, click **Edit Analysis Protocol**.



3. In the **Basecalling** tab:
   a. Select the correct Basecaller and DyeSet/Primer files in the drop-down lists.
   b. If you want, select one or more stop points for data analysis.
4. Do the following:

If you …	Then …
Want to make additional changes to the Analysis Protocol	Complete steps 5 to 8.
Are done making changes	Go directly to step 8.

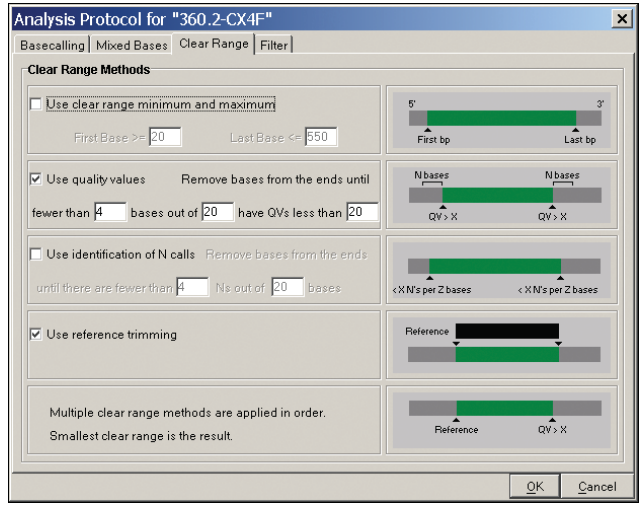
5. Select the **Mixed Bases** tab.



a. If desired, select **Use Mixed Base Identification**.

b. Use the default detection level of 25% or change the it by entering a new value or by dragging the % line up or down.

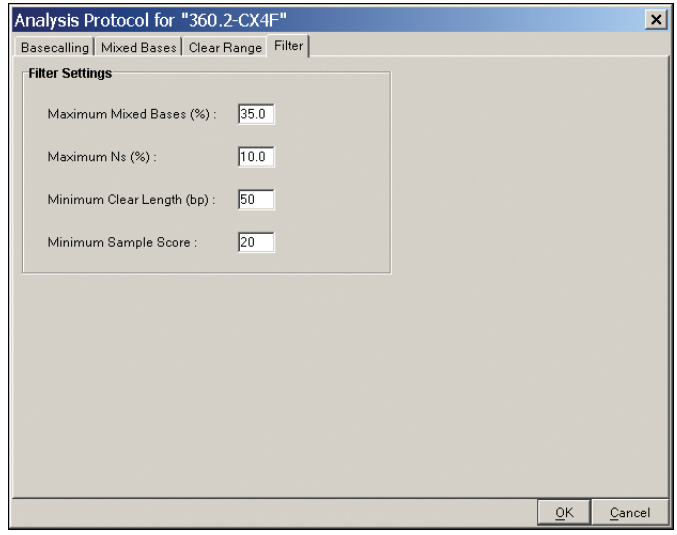
6. Select the **ClearRange** tab.



a. If desired, select one or more stop points for data analysis.

b. Select **Use reference trimming**.

7. Select the **Filter** tab.



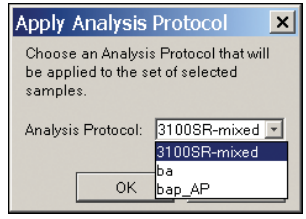
Specify the values for the filter Settings.

8. Click **OK** to save the protocol and close the Analysis Protocol dialog box.

Applying the Analysis Protocol

To apply an analysis protocol:

1. Select the samples in the Sample Manager to apply the new settings to.
2. Click **Apply Analysis Protocol**.



3. Select a protocol from the Analysis Protocol drop-down list.
4. Click **OK**.

   The spacing, peak 1, and start and stop points change to zero.
5. Click **Apply**.

   The Assembled indicator changes from green (assembled) to red (unassembled), and the Analysis button becomes active.
6. Click  .

Editing the Data

About Sequence Editing

To edit a sequence, you can:

- Adjust the clear range
- Add, delete, or change a base in a sample
- Add or delete a space in a sample
- Add, delete, or change a base in a specimen consensus
- Add or delete a space in a specimen consensus
- Add or delete a space in a reference

You can edit sequences within a project. The change is immediately reflected in the consensus sequence. You can also edit the consensus sequence. In this case, all the samples change to reflect the consensus edits. You can edit consensus sequences when viewing the data in the Specimen view or in the Project view.

Note: An edited base change or insertion appears in lowercase to distinguish it from an unedited base. This applies to both user edits and consensus-caller edits. See "Editing Bases with Quality Values" on page 10-10 for more information on editing bases with QVs.

When to Edit the Data

After analysis is complete, and you generate the analysis reports, depending on the results in the reports, you may want to:

- Adjust the clear range for a sample (see "Adjusting the Clear Range" on page 8-14)
- Edit a base or space in a sample or specimen (see "Editing a Sample or a Consensus Sequence" on page 8-12)

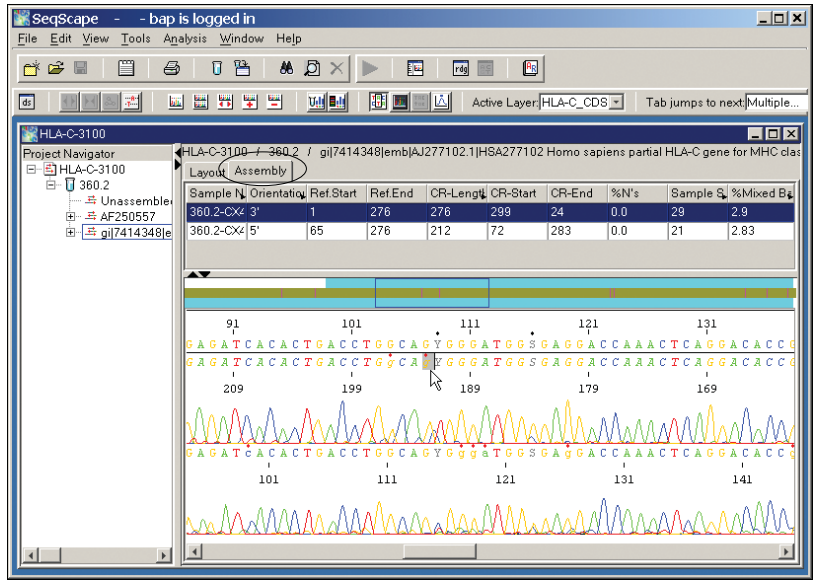
Editing a Sample or a Consensus Sequence

Editing a Consensus Sequence in the Segment View

To insert, delete, or change a base in the Specimen view:

1. Select the Segment icon in the navigation pane in the Project window.

   The Specimen view opens in the project document window.



2. Select the **Assembly** tab, then select a layer in the Active Layer drop-down list.
3. To change or delete a consensus base, click the base you want to edit, then delete or change the base.
4. To insert a base in the consensus sequence, click between two bases, then insert the bases.

Note: The changed bases appear in lowercase.

Note: If the audit feature is enabled, you must enter a reason for each base change, base insertion, and base deletion.

Editing Sample Bases

You can edit sample bases in the same manner as consensus bases. However, only the sample whose base is edited and the consensus sequence are affected by the changes.

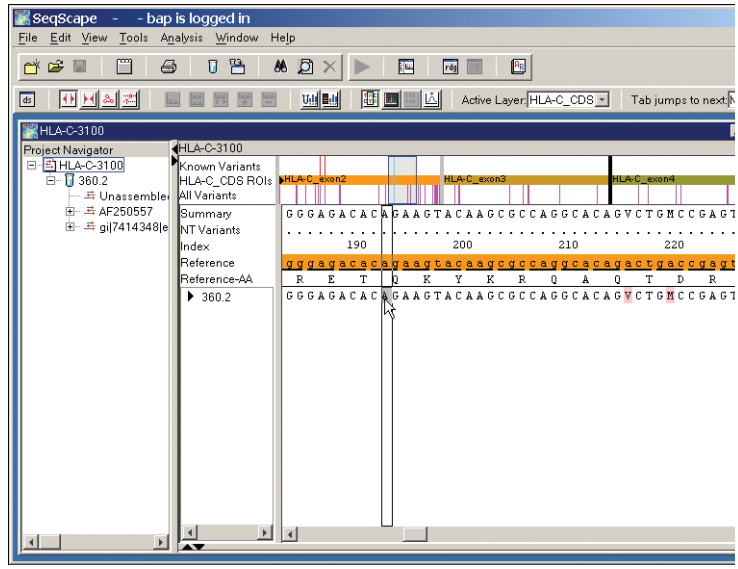
Editing a Consensus Sequence in the Project View

Note: Any changes are reflected in the sample sequences within the specimen and in the summary.

To insert, delete, or change a base in the Project view:

1. Select the project of interest in the navigation pane in the Project window.

   The project view opens displaying the consensus sequences for each specimen.



2. To change or delete a consensus base, click the base you want to edit, then delete or change the base.
3. To insert a base in the consensus sequence, click between two bases, then insert the base.

   Note: The changed bases appear in lowercase.

4. To delete a space, click the space to select, then press the **Delete** key.
5. To insert a space, click where you want to insert a space, then press the dash key or space bar.

Note: If the audit feature is enabled, you must enter a reason for each base change, base insertion, and base deletion.

Adjusting the Clear Range

About the Clear Range

Sample data usually has unreadable or otherwise unusable sequence located at the beginning and end of the data. Inclusion of this data causes errors in the alignments and erroneous variant detection.

The clear range is the area of continuous sequence that is the most error free. In the SeqScape software, the clear range is set automatically for all samples during the analysis based on the Analysis Settings for that sample. You can modify the clear range on a per-sample basis.

IMPORTANT! If you do not select Use Reference Trimming in the Analysis Settings, you should manually set the clear range to remove any sample data that lies 5′ of the 5′end or 3′of the 3′end of the reference, if needed. Any sample data that is outside the reference is not aligned.

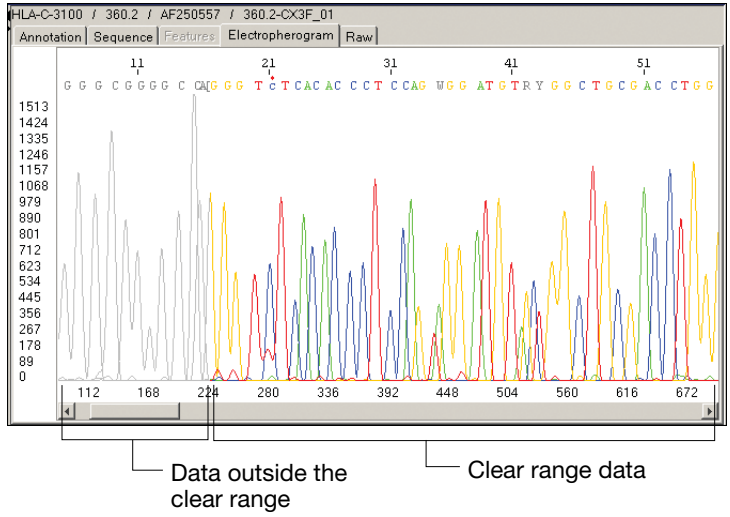


Figure 8-3 Clear Range Data

After changing the clear range, the specimen is automatically reassembled, then realigned and recompared to the reference.

The three methods for changing the clear range involve using the:

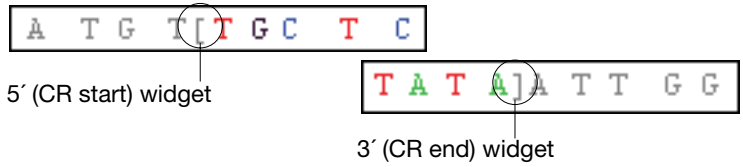
- Clear range widgets
- Mouse
- Set Clear Range dialog box

Using the Clear Range Widget

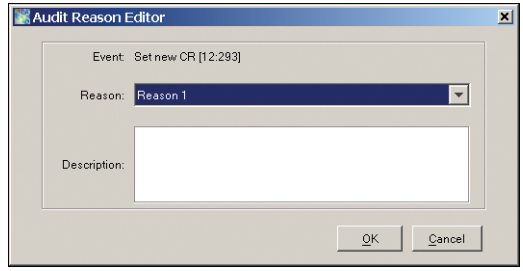
To use the Clear Range widget to adjust the clear range:

1. Open a sample from within a project.
2. Select the **Electropherogram** tab.
3. Locate and select the 5′ (CR start) or 3′(CR end) widget.

   The widget turns from gray to black, when selected.



4. Drag the widget along the bases to the right or left, as desired, then release the cursor.
5. If the audit feature is enabled, an Audit Reason Editor opens.



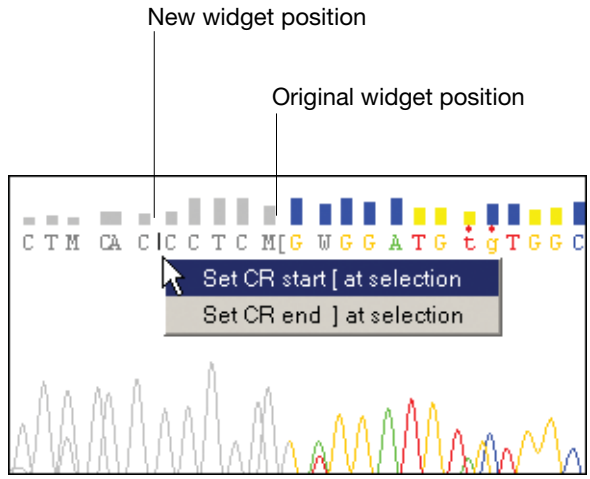
6. Complete the Audit Reason Editor dialog box, then click **OK**.

   The new clear range is displayed.
7. Repeat the process to define a new clear range for the opposite end.

Using the Mouse

To use the mouse to adjust the clear range:

1. Open a sample from within a project
2. Select the **Electropherogram** tab.
3. Right-click between two bases where you want to move the 5′ (CR start) or 3′(CR end) widget.



4. Do one of the following:

If you are moving the…	Then select…
CR start widget	**Set CR start [at selection**
CR end widget	**Set CR end] at selection**

5. If the audit feature is enabled, select a reason in the Audit Reason Editor, then click **OK**.

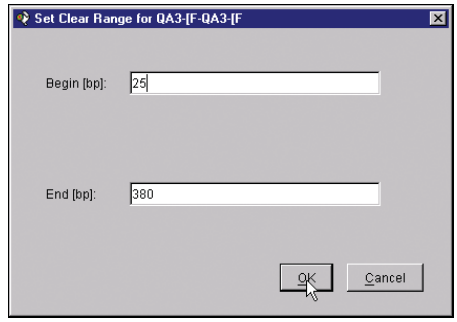
   The new clear range is displayed.

6. Repeat the process to define a new CR widget position for the opposite end.

Using the Set Clear Range Dialog Box

To use the dialog box to adjust the clear range:

1. Open a sample from within a project.
2. In the Electropherogram view or Specimen view, determine your new beginning and ending base numbers.
3. Select **Tools > Set Clear Range**.



4. Enter the values determined in step 2, then click **OK**.
5. If the audit feature is enabled, select a reason in the Audit Reason Editor, then click **OK**.

   The new clear range is displayed.

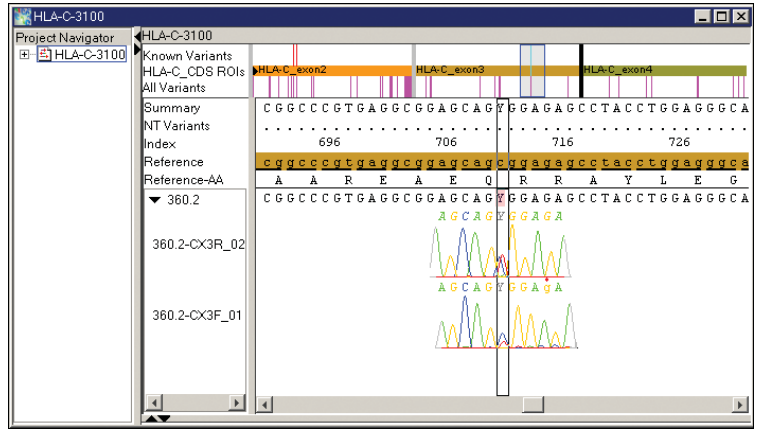
Editing Variants

After you clean up errors in the sequences, you can view and edit the variants. Two methods to review variants follow.

Method 1 To view and edit variant data:

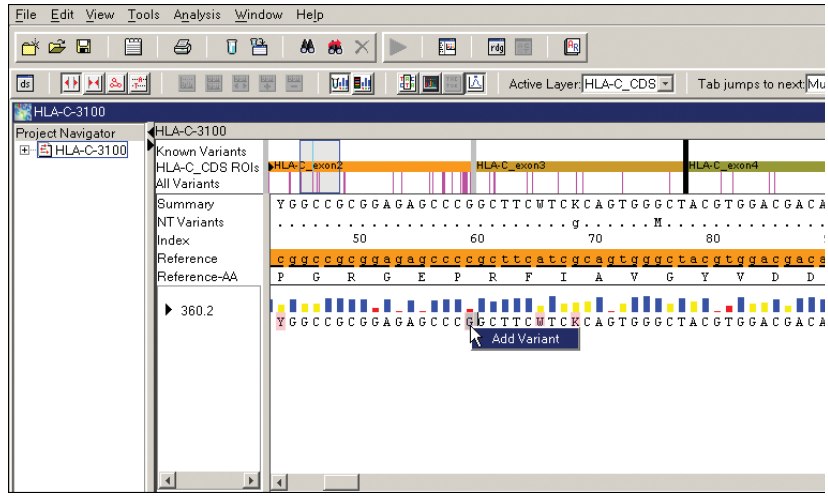
1. Open a project of interest.
2. Click a summary base.
3. Click the triangle that appears next to the specimen name to view the electropherogram snippets.
4. Edit bases or spaces in the specimen consensus sequences or the shown sample data.
5. Press **Tab** to move to the next variant or press **Shift-Tab** to move to the previous variant to view or edit more positions.

   Note: Pressing Ctrl+Z centers the selected column in the display, even if snippets are not showing.



Method 2 To view and edit variant data:

1. Open the project of interest.
2. Select **Analysis > Report Manager**.
3. In the navigation pane, select the report you want to view.
4. Select **Window > Tile**.
5. Review the positions by selecting a base change in the Mutations table. This adjusts the alignment view to the correct position in the alignment.
6. To add an unknown variant to the RDG, right-click the unknown variant position in a consensus sequence in the project alignment, then click **Add Variant**.



Saving Your Data

When you finish, save your project. Select **File > Save Project** or click (Save Project).

Exporting and Printing Data and Reports

9

This chapter contains:

Workflow for This Chapter

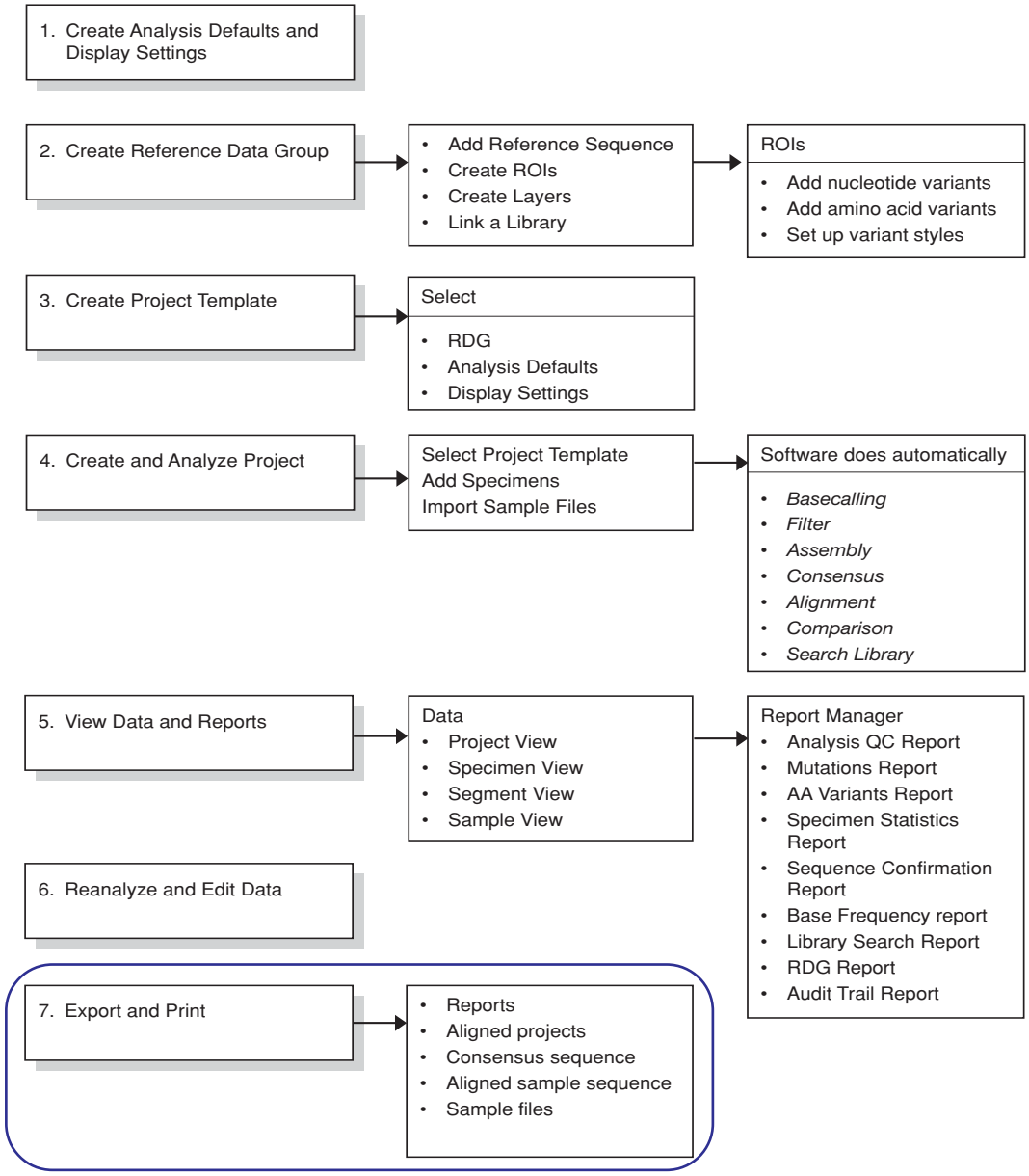


Figure 9-1 Showing the Printing and Exporting Steps

Exporting Data Files

File Names

The default file name uses the project name and the report type.

Do not use the following characters in any file name:
\ / : * ? “ < > | & and space

Format Options

You can export a project, specimen, segment, or sample file. Table 9-1 summarizes the available format options. Header and footer information is not incorporated in any data file.

Note: Only one data file can be exported at a time.

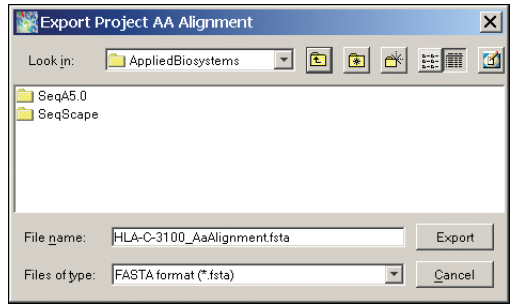
Table 9-1 Export and File Format Options

Export Option	File Format Options
Project	
Project Alignment-Nucleotides	FASTA
Project Alignment-Amino Acids	
Specimen and Segment	
Consensus Sequence	FASTA, SEQ, or QUAL
Aligned Sample Sequence	FASTA
Sample	
Sample Sequence File	FASTA, SEQ, AB1, or PHD

Exporting a Project Alignment

To export a project alignment:

1. Open the project of interest.
2. In the navigation pane, select the project icon.
3. Select **File > Export > Project Alignment-Nucleotides** or **Project Alignment-Amino Acid**.



4. Complete the Export Project dialog box:
   a. Select a folder location to store the project view.
   b. Change the file name, if desired.

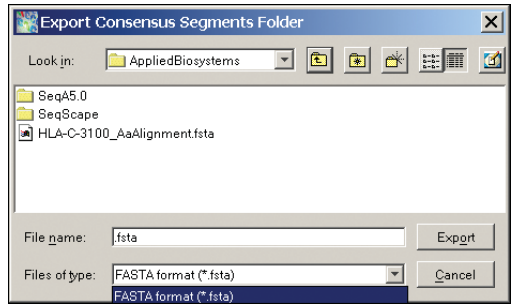
   Note: The default file name uses the project name with the element type suffix and the FASTA extension.

   c. Click **Export**.

Exporting a Specimen

To export a specimen:

1. Open the project of interest.
2. In the navigation pane, select a Specimen icon.
3. Select **File > Export > Consensus Sequence** or **Aligned Sample Sequence**.



4. Complete the Export Consensus dialog box:

   a. Select a folder location to store the file.

   Note: The default file name uses the project name with the element type suffix and the FASTA extension.

If the number of segments in a project is …	Then …
One	Change the file name, if desired.
Two or more	Do *not* type a file name. **Note:** The individual segment names are used. Any name you type is ignored.

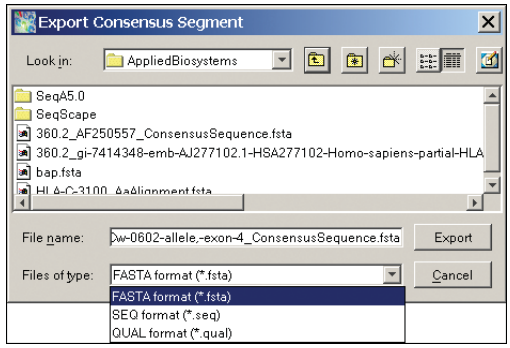
   b. For the Consensus Sequence option, select a file format in the Files of type drop-down list.

   c. Click **Export**.

Exporting a Segment

To export a segment:

1. Open the project of interest.
2. In the navigation pane, select a Segment icon.
3. Select **File > Export > Consensus Sequence** or **Aligned Sample Sequence**.

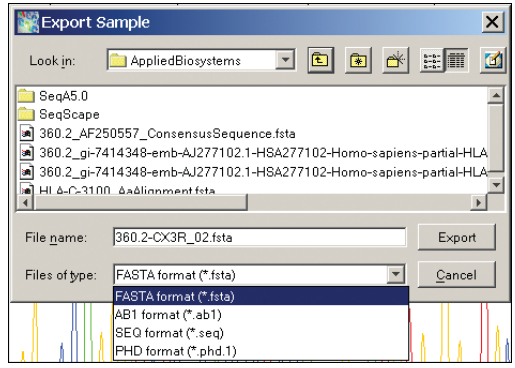


4. Complete the Export dialog box:
   a. Select a folder location to store the file.
   b. Change the file name, if desired. The default file name uses the segment name and the FASTA extension.
   c. For the Consensus Sequence option, select a file format in the Files of type drop-down list.
   d. Click **Export**.

Exporting a Sample

To export a sample:

1. Open the project of interest.
2. In the navigation pane, select a Sample icon.
3. Select **File > Export > Sample Sequence File**.



4. Complete the Export dialog box:
   a. Select a folder location to store the file.
   b. Change the file name, if desired. The default file name uses the sample name and the FASTA extension.
   c. Select a file format in the Files of type drop-down list.
   d. Click **Export**.

Exporting Reports

File Names

The default file name uses the project name and the report type.

Do not use the following characters in a file name:
\ /: * ? > | and space

Format Options

You can export generated reports as portable document format (pdf), text, HTML, or XML (Table 9-2).

Note: When selecting between HTML and XML, use HTML for standard display and XML for scripting applications.

Table 9-2 File Formats and Corresponding Application Options

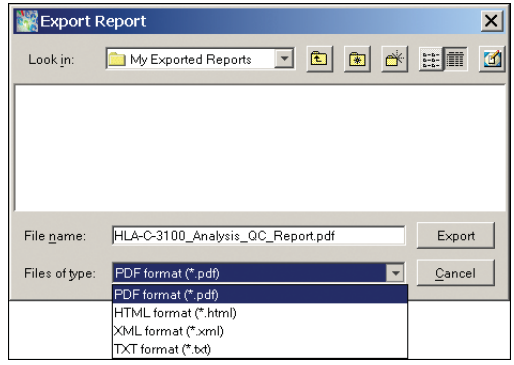
File Format	Open with…
PDF (default)	Adobe® Acrobat® Reader™
HTML*	A web browser or any software that is able to display HTML files
XML	A web browser
TXT	Notepad, Wordpad, Microsoft® Word, or any text-compatible software

*When exporting the report as HTML, a folder is automatically created that may contain more than one HTML file. The file that uses only the report name contains all the data from the report.

Exporting a Report

To export a report:

1. Open the project of interest, then click  .
2. In the navigation pane, select a report type.
3. Customize the report, if desired. (See "Customizing the Reports" on page 7-36.)
4. Select **File > Export > Report**.



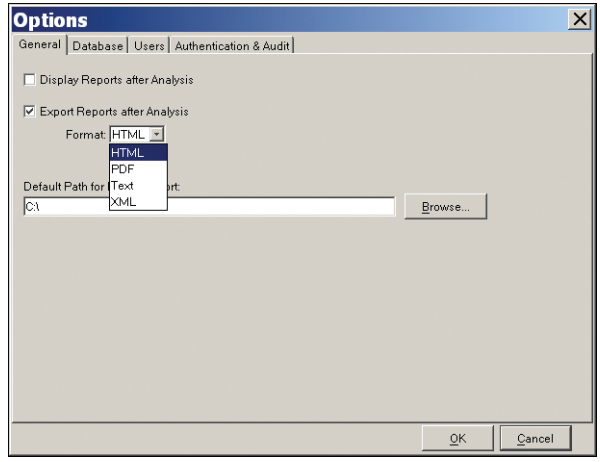
5. Complete the Export Report dialog box:
   a. Select a folder location to store the report.
   b. Change the file name of the report, if desired. The default file name uses the project name, the report type, and the .pdf extension.
   c. Select a file format in the Files of type drop-down list.
   d. Click **Export**.

Exporting All Reports Automatically

Reports can be automatically exported after analysis.

To set up for automatic exporting of reports:

1. Select **Tool > Options**.



2. Complete the General tab of the dialog box:
   a. Select the **Display Reports after Analysis** check box, if desired.
   b. Select the **Export Reports after Analysis** check box, then select an export format from the drop-down list.
   c. Define a default location to save the exported files.
   d. Click **OK**.

Printing Data and Reports

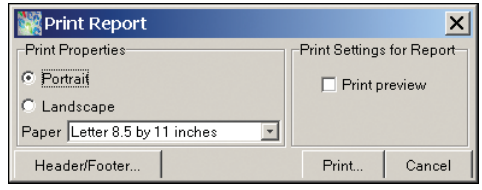
You can print any viewable screen in a WYSIWYG (what you see is what you get) manner within the SeqScape software on one of the recommended printers (HP 8100, 4500, 990cxi, and Epson 980). You can print project, specimen, segment, and sample views, as well as the reports for a project.

Customizing Header and Footer Display

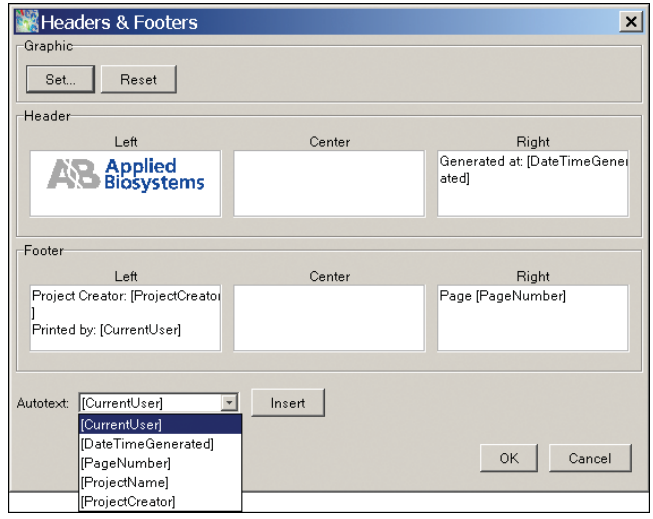
Default header and footer information is included in all exported and printed reports and in printed data views. However, headers and footers are not included in exported data files.

To customize the header/footer display in printed and exported reports:

1. Select **File > Print**.



2. Click **Header/Footer**.



3. To change the graphic, if desired:
   a. In the Graphic section, click **Set**.
   b. In the dialog box, locate then select a graphic file.
   c. Click **OK**.

   Note: The graphic is displayed in the Headers & Footers dialog box and in the upper left corner of printed or exported reports.

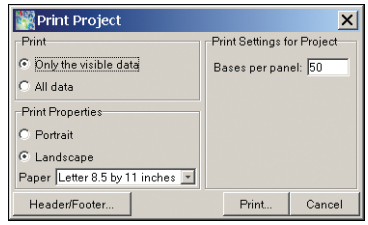
4. To change the header and/or footer information, do one of the following:
   - Type text into any of the header and/or footer text boxes.
   - Use the autotext variables from the Autotext drop-down list. (Insert the cursor in a text box, select an autotext option in the drop-down list, then click **Insert**).
   - Use a combination of typing text and using the autotext variables.

5. Save the changes:
   a. Click **OK** to close the Header & Footer dialog box.
   b. Click **Print** in Print Report dialog box.
   c. In the Print dialog box, click:
      - **Cancel** to save the changes without printing
      - **OK** to save the changes and print

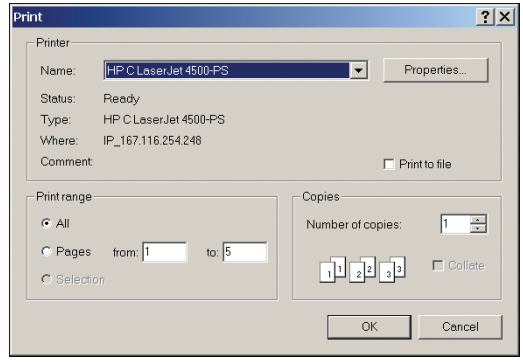
Printing Various Views of a Project

To print different views of a project:

1. Open the project of interest.
2. In the navigation pane, select a view (Project, Specimen, Segment, or Sample) to print.
3. If you are using WYSIWYG, scroll to the area of the view you want to print.
4. Select **File > Print** or click .



5. Complete the dialog box:
   a. In the Print section, select **Only the visible data** (WYSIWYG) or **All data** (if available).
   b. In the Print Properties section, select the paper orientation and size.
   c. In the Print Settings for Project section, type a new value in the Bases per panel field, if desired.
   d. Click **Print**.



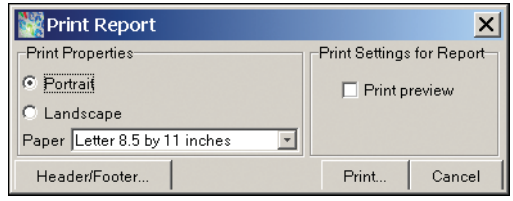
6. Select a printer, then click **OK**.

   Both print dialog boxes close and printing begins.

Printing a Report

To print a report:

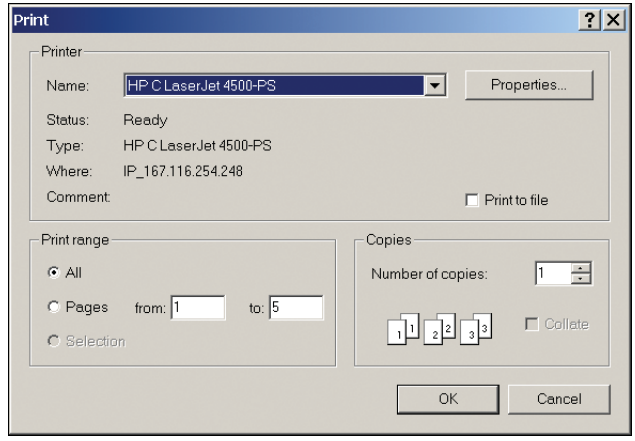
1. Open the project of interest, then click  .
2. In the navigation pane, select a report type.
3. Customize the report, if desired (see "Customizing the Reports" on page 7-36).
4. Select **File > Print**.



5. Complete the Print Report dialog box:
   a. In the Print Properties section, select the paper orientation and size.
   b. In the Print Settings for Report section, select **Print Preview**, if desired.
   c. Click **Print**.
6. Use the following table to determine your next step:

If the Print preview option was …	Proceed to step …
Not selected	7
Selected	8

7. The Print dialog box opens:



Select the printer and define the page range, then click **OK**.

8. The Report Preview dialog box opens. Use the command buttons as described in Table 9-3 on page 9-17.

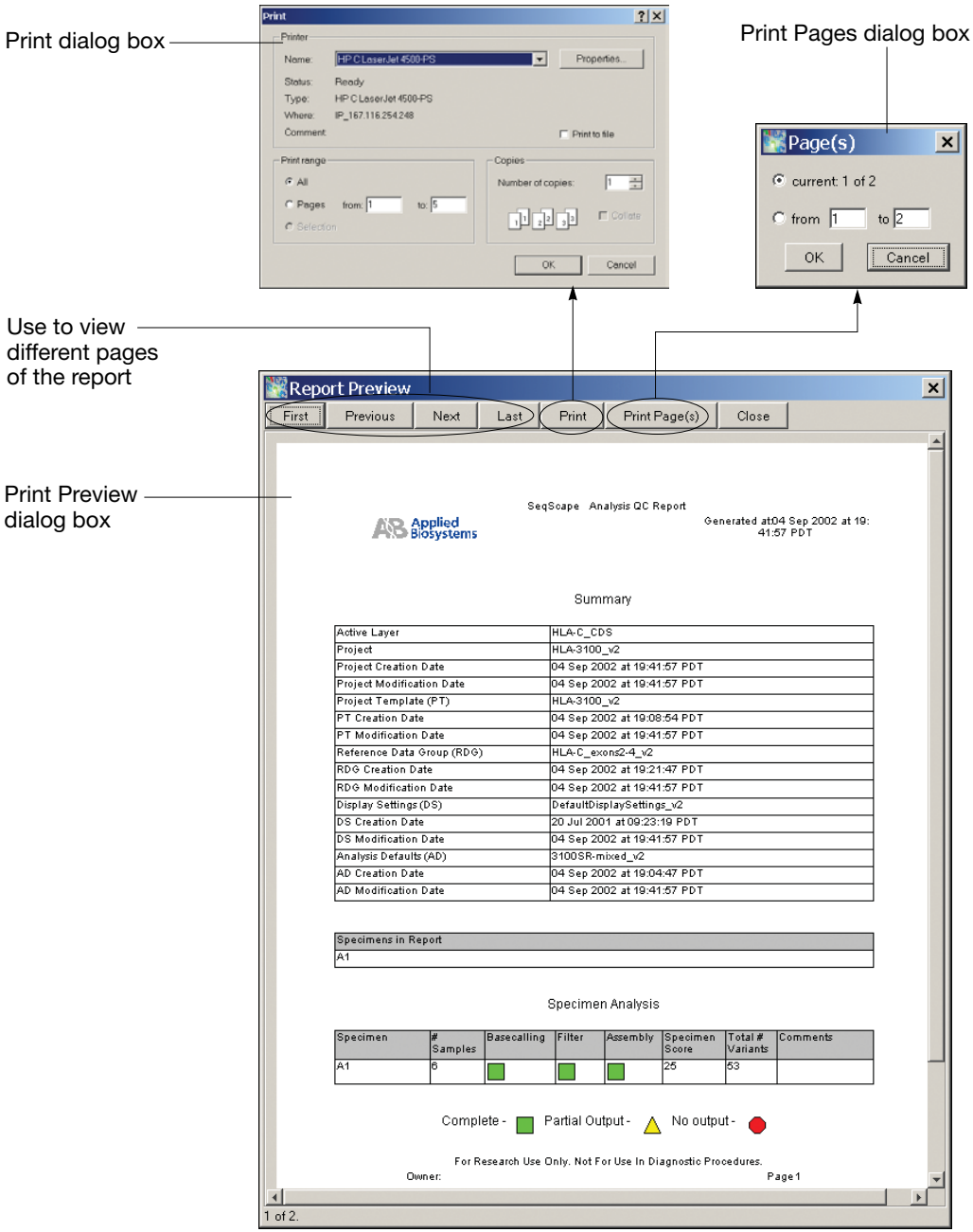


Table 9-3 Report Preview Button Functions

Button(s)	Function
First, Previous, Next, and Last	Displays the various pages in a report (only one page is visible at a time).
Print	Opens the Print dialog box. Select a printer, then click **OK** to print the report.
Print Pages	Opens the Page(s) dialog box. Set the page range, then click **OK**. In the Print dialog box, click **OK** to print the report. **Note:** Page(s) dialog box settings override the settings in the standard Print dialog box.
Close	Closes the preview window without printing the report.

Sample and Consensus Quality Values

10

This chapter contains:

Types of Quality Values (QVs)

Table 10-1 summarizes the types of QVs and where they are displayed.

Table 10-1 Quality Value Types

Quality Value Type	Definition	Location
Sample QV	A per-base estimate of basecaller accuracy.	• Sample view • Specimen view • Project view
Sample Score	The average quality value of the bases in the clear range sequence for that sample.	Specimen Statistics report
Consensus QV	A per-base estimate of the accuracy of the consensus-calling algorithm.	• Specimen view • Project view
Consensus Score	The average quality value of the bases in the consensus sequence for that specimen.	• Analysis QC report • Specimen Statistics report
Mutation QV	A per-base estimate of basecaller accuracy.	Mutations report
QV for deletion mutation	Average of the quality values for the bases to the left and right of the deletion.	Mutations report

Sample Quality Values

Sample Quality Values

A sample quality value (SQV) is a per-base estimate of the basecaller accuracy. There are two types of basecallers that generate SQVs:

- KB – A new algorithm that identifies mixed or pure bases, and generates sample quality values.
- ABI – Algorithm used in ABI PRISM® Sequencing Analysis Software v3.7 that identifies pure bases. Then the TraceTuner™ software identifies mixed bases and generates sample quality values.

KB and ABI algorithms can produce slightly different SQVs.

Interpreting the Sample Quality Values

Per-base SQVs are calibrated on a scale corresponding to:

$$QV = -10\log_{10}(Pe)$$

Where *Pe* is the probability of error of the basecall.

The range of a QV is 1 to 50, with 1 being low confidence and 50 being high confidence. See Table 10-2, "Quality Values and Probabilities of Error," on page 10-4 for the probability of basecall errors for QVs ranging from 1 to 50.

Mixed base calls yield lower SQVs than pure base calls.

Sample Score

A sample score is generated from SQVs. It is the average quality value of the bases in the clear range sequence for a sample.

Table 10-2 Quality Values and Probabilities of Error

QV	*Pe*	QV	*Pe*	QV	*Pe*
1	79%	21	0.79%	41	0.0079%
2	63%	22	0.63%	42	0.0063%
3	50%	23	0.50%	43	0.0050%
4	39%	24	0.39%	44	0.0040%
5	31%	25	0.31%	45	0.0032%
6	25%	26	0.25%	46	0.0025%
7	20%	27	0.20%	47	0.0020%
8	15%	28	0.15%	48	0.0016%
9	12%	29	0.12%	49	0.0013%
10*	10%	30*	0.1%	50*	0.001%
11	7.9%	31	0.079%		
12	6.3%	32	0.063%		
13*	5.0%	33	0.050%		
14*	4.0%	34	0.040%		
15*	3.2%	35	0.320%		
16	2.5%	36	0.025%		
17*	2.0%	37	0.020%		
18	1.6%	38	0.016%		
19	1.3%	39	0.013%		
20*	1%	40*	0.01%		

*Commonly used cut-off values for sample quality values

Consensus Quality Values

A consensus quality value (QV) is a per-base estimate of the accuracy of the consensus-calling algorithm. If the SQVs are generated from the KB basecaller, then the KB consensus-calling algorithm is used to generate the QVs. If the SQVs are generated from an ABI basecaller and TraceTuner, then the TraceTuner consensus-calling algorithm is used to generate the QVs.

The KB and TraceTuner consensus-calling algorithms can produce slightly different consensus QVs.

Interpreting the Consensus Quality Values

The degree of certainty of either consensus-calling algorithm is reflected by the per-base consensus QVs. A consensus QV is derived from a number of factors:

- How large a quality-value discrepancy exists between calls from the individual sample sequence strands
- The possible redundancy of calls from strands in the same orientation
- The possibility that the basecaller missed a mixed base

The possible values for the QVs range from 1 to 50. Higher numbers indicate calls that the algorithm determined with a measure of confidence, while lower numbers indicate calls that might require user inspection to verify the correct answer. The consensus quality values are roughly calibrated to follow the same scale as the per-base sample quality values.

Consensus Score

A consensus score is generated from consensus QVs. It represents the average quality value of the bases in the consensus sequence for a specimen.

Displaying Quality Values

QVs are displayed as bars above each base in a sample (Figures 10-1 and 10-2). The height and color of a bar indicates its value. The taller the bar, the higher the QV. The color of a bar, which is associated with its value, is editable in the Display Settings.

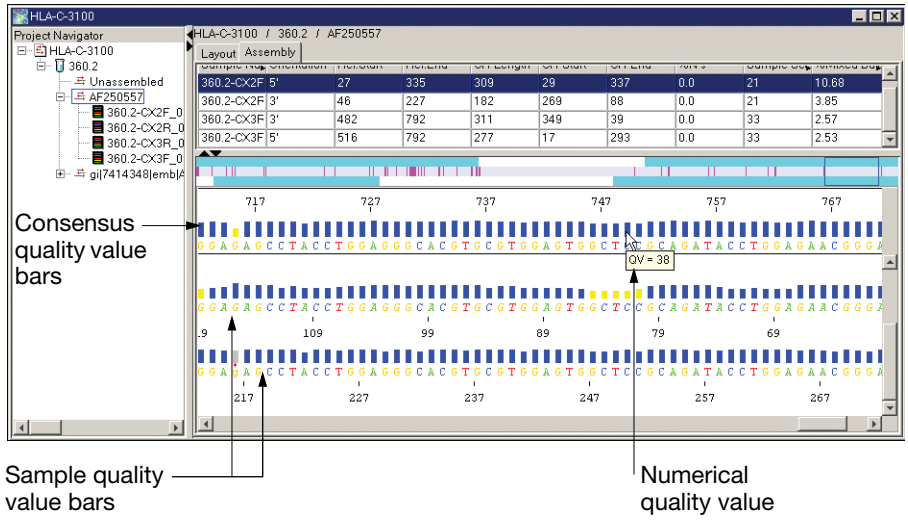


Figure 10-1 Example of QV Bars in the Specimen View

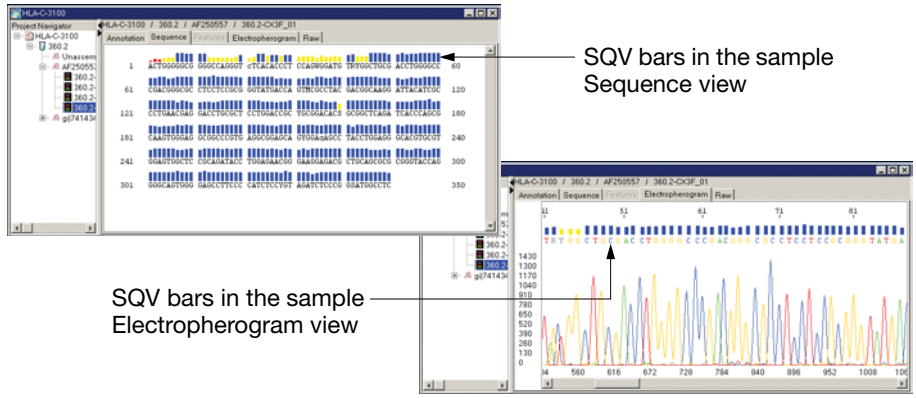


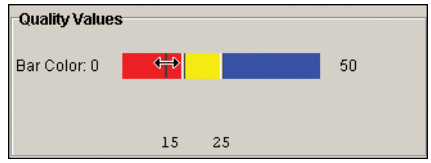
Figure 10-2 Examples of SQVs in the Sample View

Customizing the Quality Value Display

You can modify the low, medium, and high ranges and the color associated with a QV.

To modify the QV display:

1. Select **Analysis > Display Settings** or click  .
2. Select the **Bases** tab.
3. In the Quality Values section, place the pointer between two colors (it becomes a double-headed arrow), then click the slider on the color bar and drag it to left or right to the desired value.



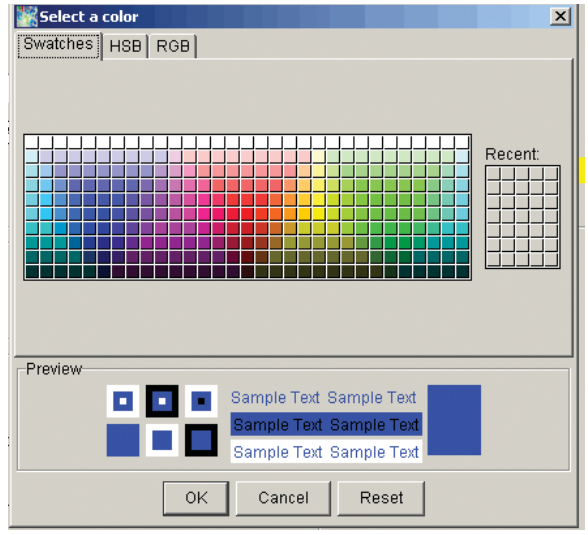
Use the criteria in the table below to define what values represent low, medium, and high ranges for your project.

QV Bar	Default Color and Range	Set the range to identify data that is …
Low	Red 0 to 14	Not acceptable
Medium	Yellow 15 to 24	Needs manual review
High	Blue 25 to 50	Acceptable

4. Change the colors that represent low, medium, and/or high QVs, if desired:

   a. Select the color in the Bar Code you want to change.

      The Select a color dialog box opens.



   b. Select a new color in the Swatches tab, or use the HSB or RGB tabs to define a new color.

   c. Click **OK**. The color dialog box closes.

5. Click **OK.**

Displaying the Quality Bars and Values

If you do not see the QV bars when viewing samples or a consensus in a project, then follow the procedures below to display QV bars and values.

To view quality bars and values:

1. Open a project, then open a specimen of interest.
2. Select the segment of interest, then select the **Assembly** tab.
3. To view sample QVs, select **View > Show/Hide sample QV** or click  .
4. To view consensus QVs, select **View > Show/Hide consensus QV** or click  .
5. To obtain a numerical value for a particular bar, place the cursor over the bar for 2 sec. The value is automatically displayed.

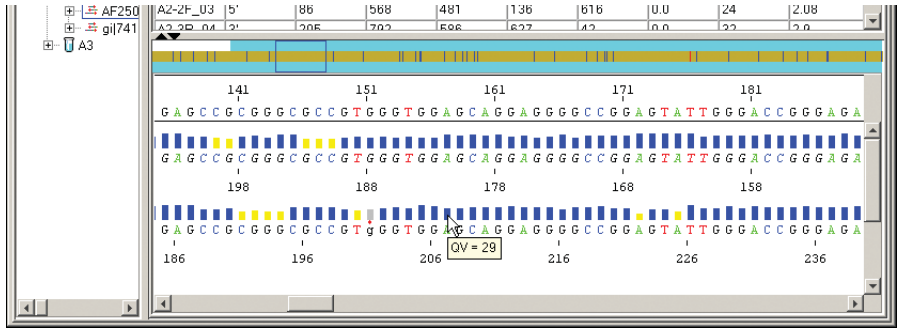


Figure 10-3 Displaying the Value of a Sample QV Bar

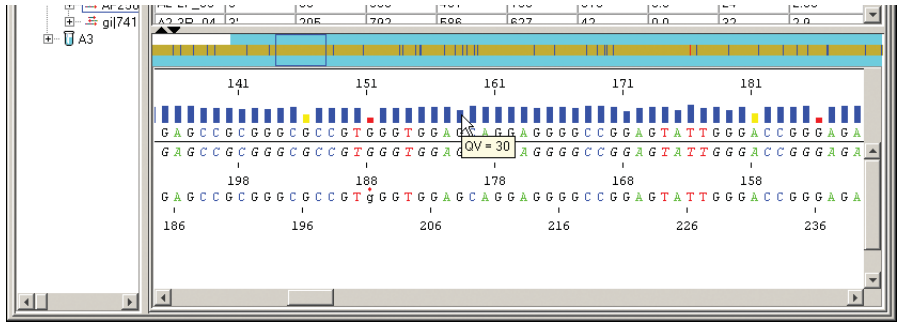


Figure 10-4 Displaying the Value of a Consensus QV Bar

Editing Bases with Quality Values

Changing, deleting, and inserting a base affect the consensus or sample QVs displayed.

Table 10-3 Results of Editing Bases with Quality Values

If …	Then …
The consensus-caller calls a base not present in all the samples	The new base is in uppercase in the consensus sequence and in lowercase in the samples that did not contain that basecall with a red dot.
You change a base	The new base is in lowercase and the SQV has the same value but is displayed as a gray bar.
You change a base back to the original call	The base appears in uppercase and the quality value bar color is restored.
You insert a base	The inserted base appears in lowercase and it has no SQV.
You delete a base	The quality value for the base disappears.
You reinsert a deleted base	The reinserted base appears in lowercase and it has no SQV.

Cumulative Quality Value Scoring in Reports

Quality values and scores are also displayed in several reports. To view the reports, select **Analysis > Report Manager** or click .

Analysis QC Report

Consensus scores in an Analysis QC report are shown as an average quality value across the consensus sequence for each specimen.

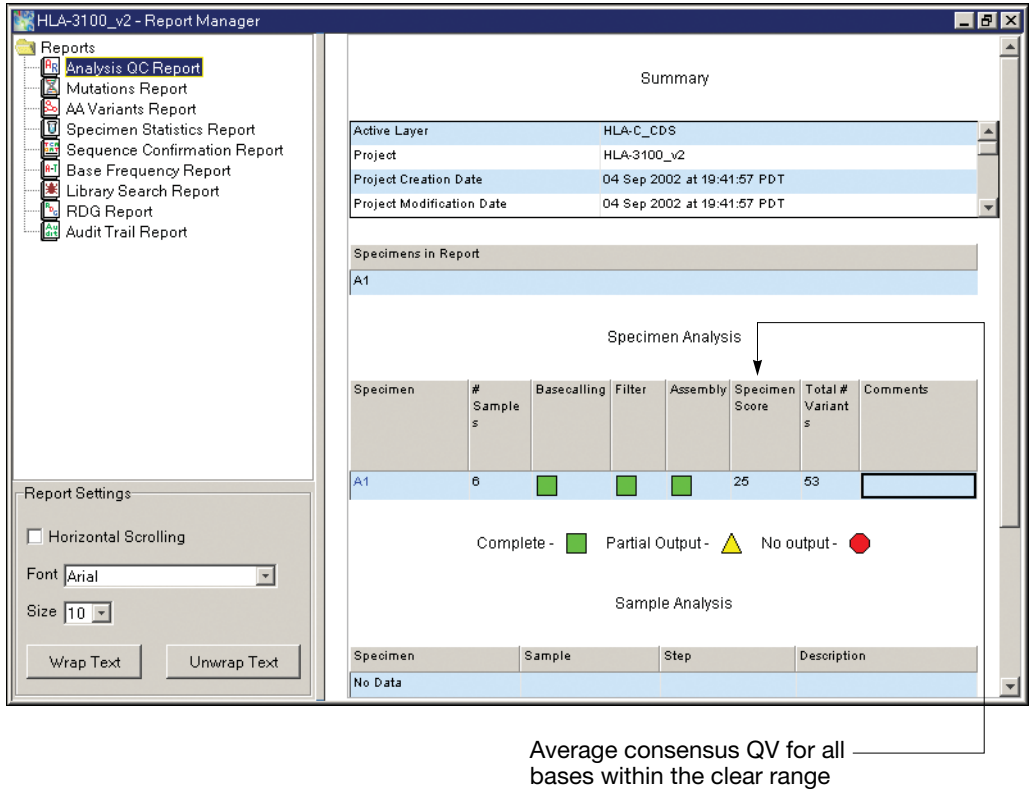


Figure 10-5 Analysis QC Report

Mutations Report

QVs for each mutation, and the average QV for the bases to the left and right of the deletion are provided in Mutations report.

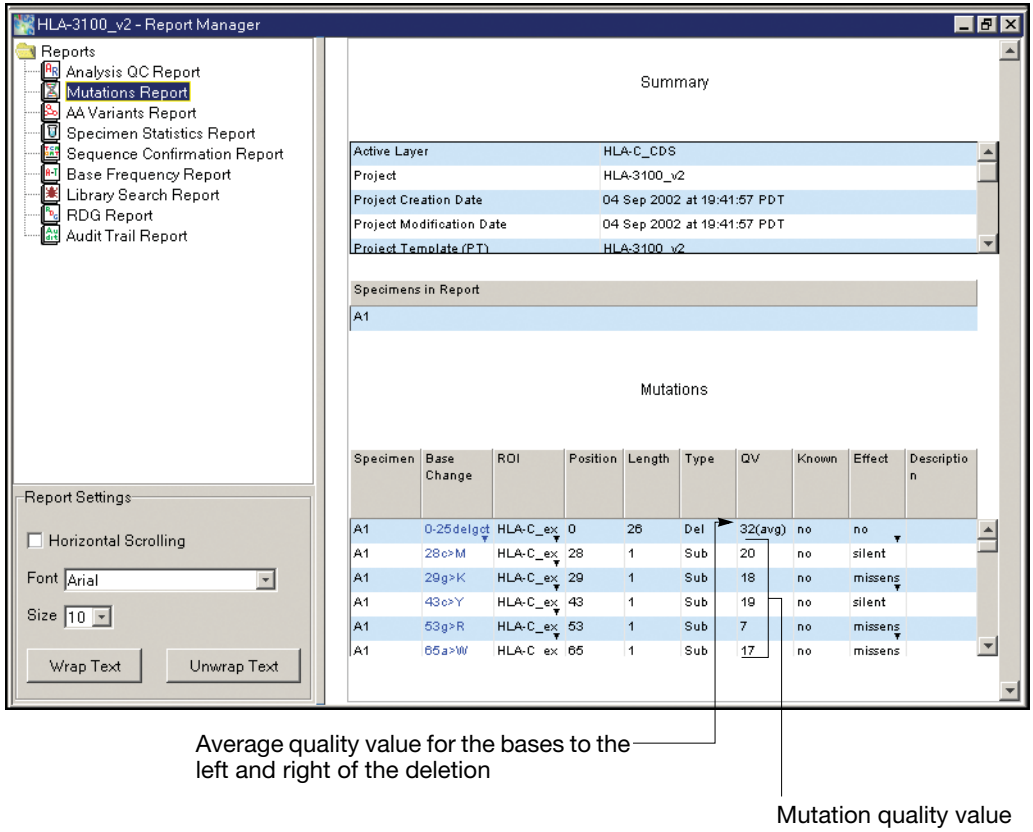


Figure 10-6 Mutations Report

Specimen Statistics Report

The Specimen Statistics table of this report displays the average consensus QV score for a segment in the Segment Score column.

The Sample Results table displays the average sample QV for the bases in the clear range in the Sample Score column.

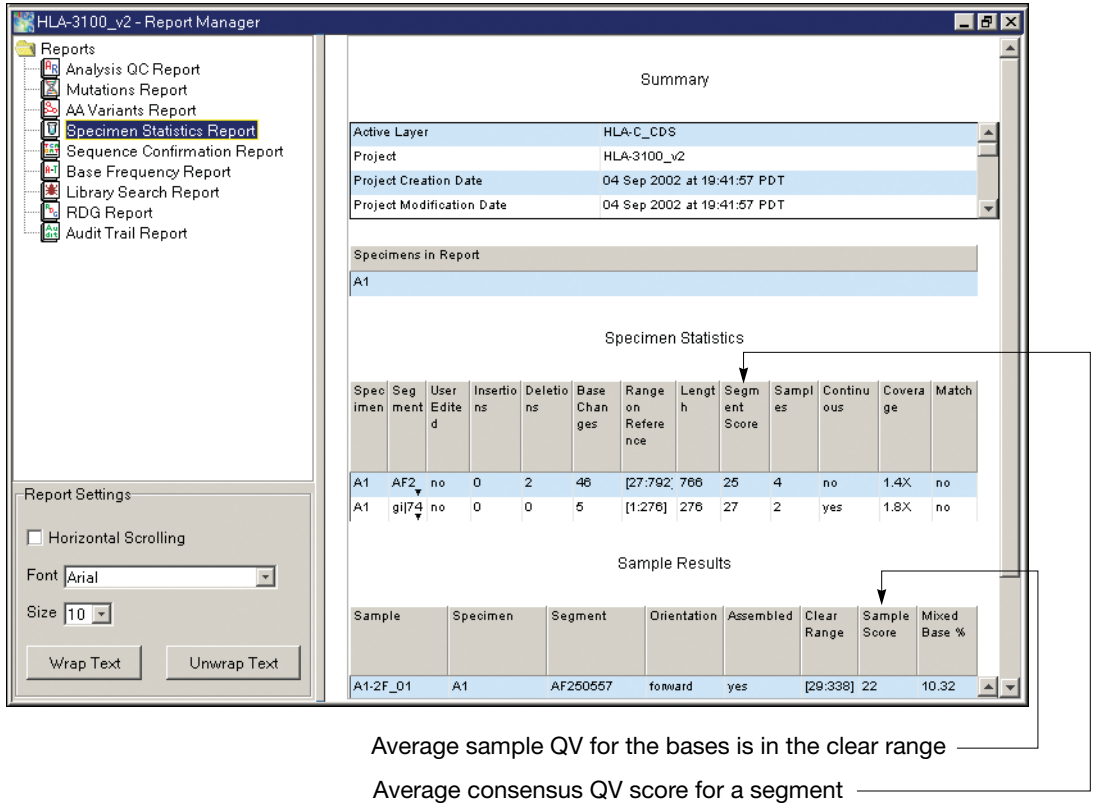


Figure 10-7 Specimen Statistics Report

Automating Analysis 11

This chapter contains:

Integrating SeqScape and Data Collection Software

Overview

Sequencing data that is generated on the Applied Biosystems 3730/3730*xl* DNA Analyzers can be automatically analyzed for use in the ABI PRISM® SeqScape Software v2.0. Autoanalysis can be performed only on the same instrument computer that collected the sample files. You can configure the software packages to perform data collection and then data analysis without requiring user interaction.

Autoanalysis requires three software packages:

- 3730/3730*xl* Data Collection software

  The data collection software is used to run the instrument and collect fluorescent data from samples. For autoanalysis to occur, the software must be set up properly to allow communication with downstream software.

  Data collection software uses a data service. Data used for data collection as well as that created in SeqScape software can be accessed through the data service in data collection software.

- Autoanalysis Manager

  The Autoanalysis Manager is software that is part of the integration between the data collection, SeqScape, and ABI PRISM® GeneMapper™ software. It can queue messages and track the status of their processing. Each message is considered a batch job, whether it contains a single sample, samples from a result group, or an entire run of samples.

  Autoanalysis Manager is installed by Seqscape or GeneMapper software when loaded on a system with data collection software.

- A version of SeqScape software with no user interface

  This version of SeqScape is identical to the regular version of the software except that no user interface exists. The Autoanalysis Manager opens and uses this version of software to analyze the data in the projects.

  The automated processing version and the standard version of SeqScape software are installed from the SeqScape Software installation CD.

IMPORTANT! When installing SeqScape software v2.0 on a computer that is connected to a 3730/3730*xl* DNA Analyzer, the data collection software must be running. If data collection software is not running, the SeqScape software does not register with the Data Service. See Chapter 2, "Installing the SeqScape Software," for information on properly installing the software.

IMPORTANT! After the initial installation of the SeqScape software, you must open the Autoanalysis Manager software.

Software Relationships

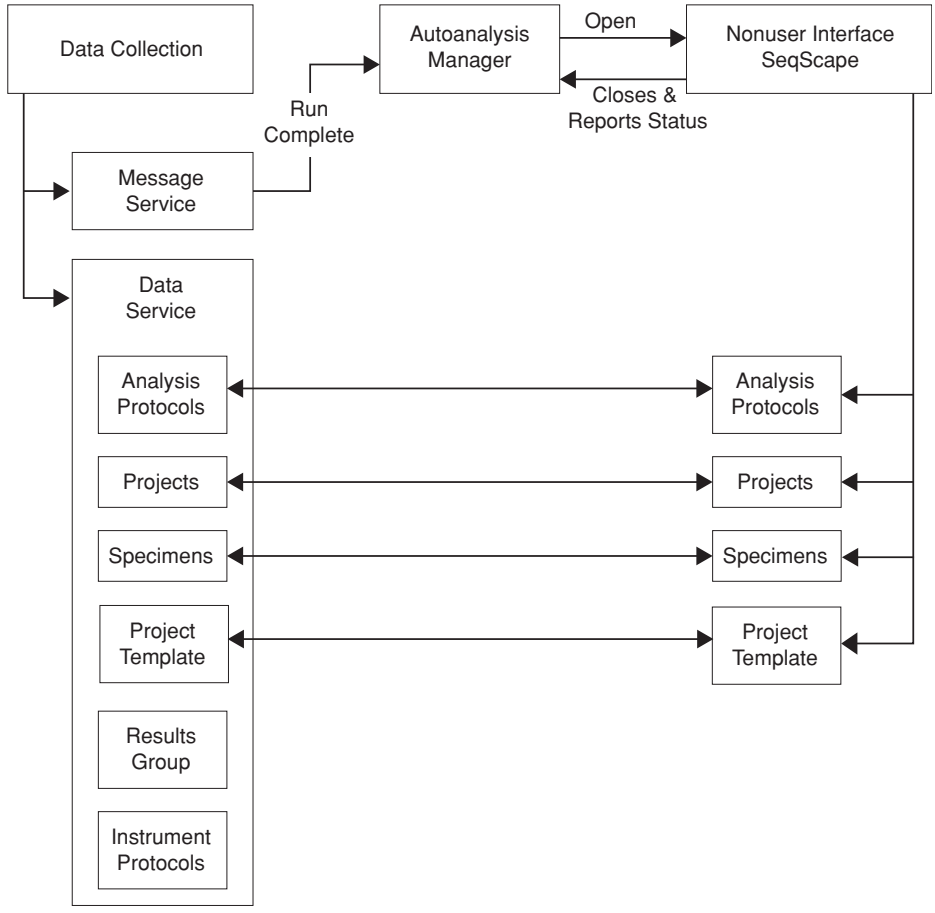


Figure 11-1 Relationships Between Seqscape and Data Collection Software

Before You Start

Successful automatic analysis requires that the:

- SeqScape software is installed properly
- SeqScape software is registered and the appropriate user IDs have been created
- Autoanalysis Manager software is running
- The 3730 instrument is set up to run, and samples are prepared

  For more information on setting up and using the 3730 Data Collection software, refer to the *Applied Biosystems 3730/3730xl User Guide* (PN 4331468).
- Files for a data collection software plate record are available

  For data collection and autoanalysis to be successful, each run of samples must have an instrument protocol, an analysis protocol, and a results group assigned within a plate record.

  The table below describes what each file specifies in the logical order of its use.

Table 11-1 File Specifications

File	Description	Created in
Instrument Protocol	Contains everything needed to run the instrument.	Data collection software
Analysis Protocol	Contains everything needed to analyze sequencing data.	Data collection software or SeqScape software
Results Group	Defines the file type, the file name, file save locations, default analysis protocols linked to sample injections, and user name and password.	Data collection software

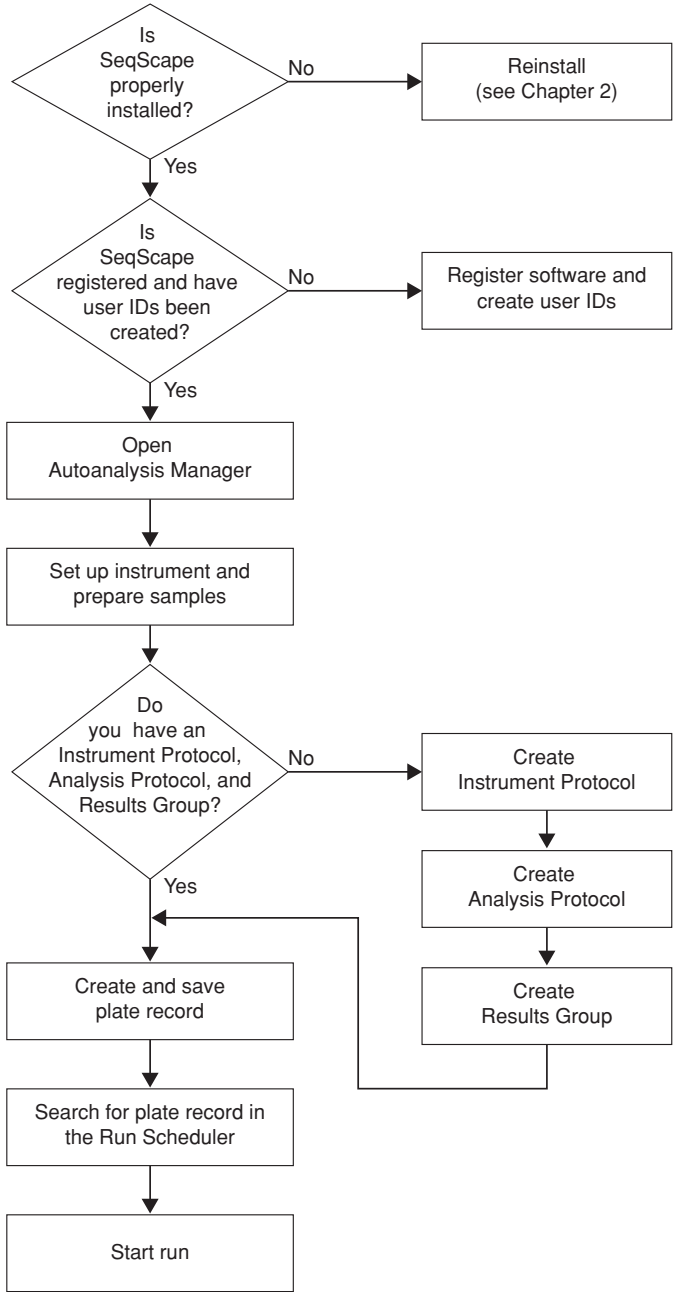


Figure 11-2 Workflow for Autoanalysis

Creating Required Files in the Data Collection Software

For More Information

For more information on setting up and using the 3730/3730*xl* DNA Analyzer and/or 3730 Data Collection software, refer to the *Applied Biosystems 3730/3730xl User Guide* (PN 4331468).

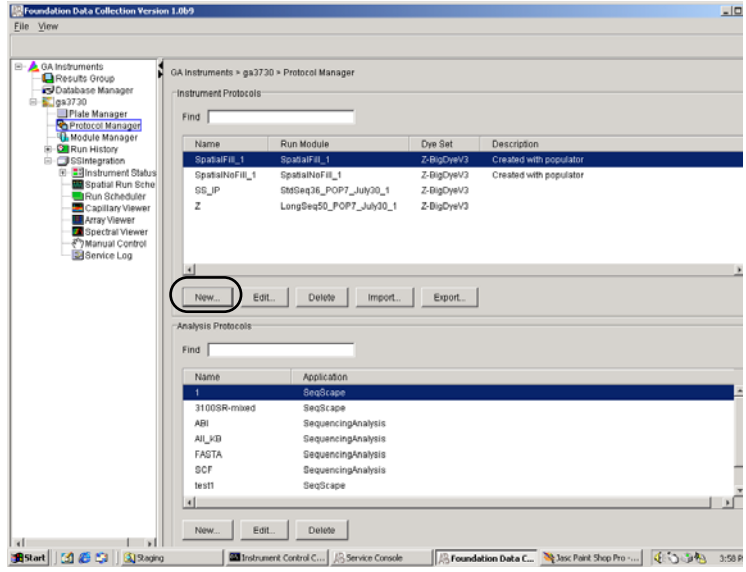
If the Files Already Exist

If the appropriate instrument protocol, analysis protocol, and results group have been created, proceed to "Creating a Plate Record" on page 11-16.

Creating an Instrument Protocol

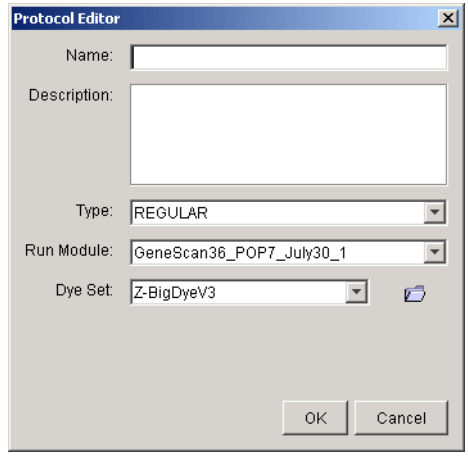
To create an instrument protocol:

1. In the navigation pane, click the **Protocol Manager** icon to display the Protocol Manager.



2. In the Instruments Protocols section, click **New** or select an existing instrument protocol file, then click **Edit**.

   The Protocol Editor opens.



3. Complete the Protocol Editor:
   a. Type a name for the protocol.
   b. Type a description for the protocol (optional).
   c. Select **Regular** in the Type drop-down list.
   d. Using the information in the table below, select the correct run module for your run.

Sequencing Analysis Type	Capillary Array Length	Run Module
Long DNA	50 cm	LongSeq50_POP7
Standard read DNA	36 cm	StdSeq36_POP7
Rapid read DNA	36 cm	RapidSeq36_POP7

e. Using the information in the table below, select the correct Dye Set for your run.

Sequencing Analysis Type	Capillary Array Length	Dye Set
Long read DNA	50 cm	Z-BigDye v3
Standard read DNA	36 cm	Z-BigDye v3
Rapid read DNA	36 cm	Z-BigDye v3

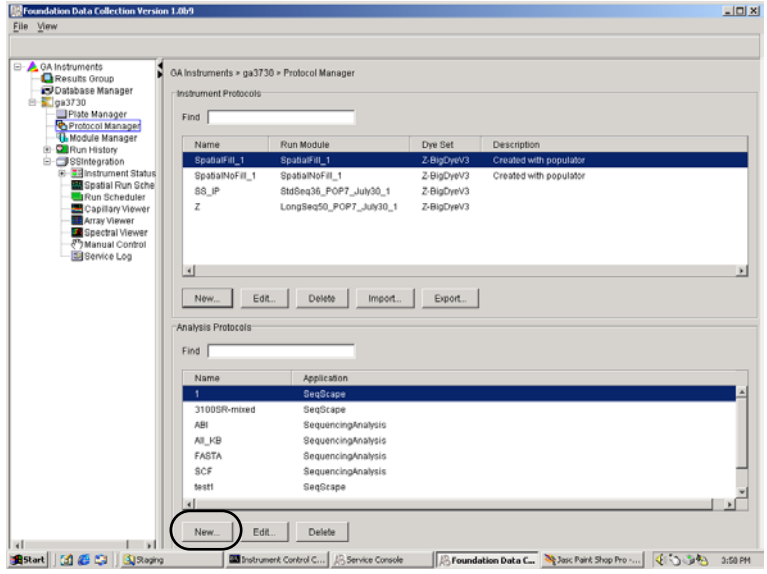
f. Click **OK**.

Creating an Analysis Protocol

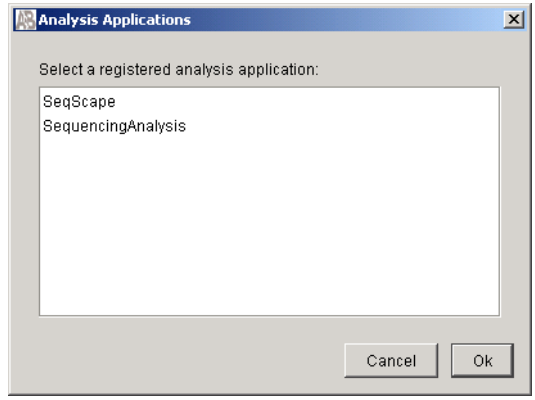
IMPORTANT! If you created an appropriate analysis protocol in SeqScape software, you can use it in data collection software. You can also create an analysis protocol in the SeqScape software, if desired.

To create an analysis protocol:

1. In the Analysis Protocol section of the Protocol Manager, click **New**.

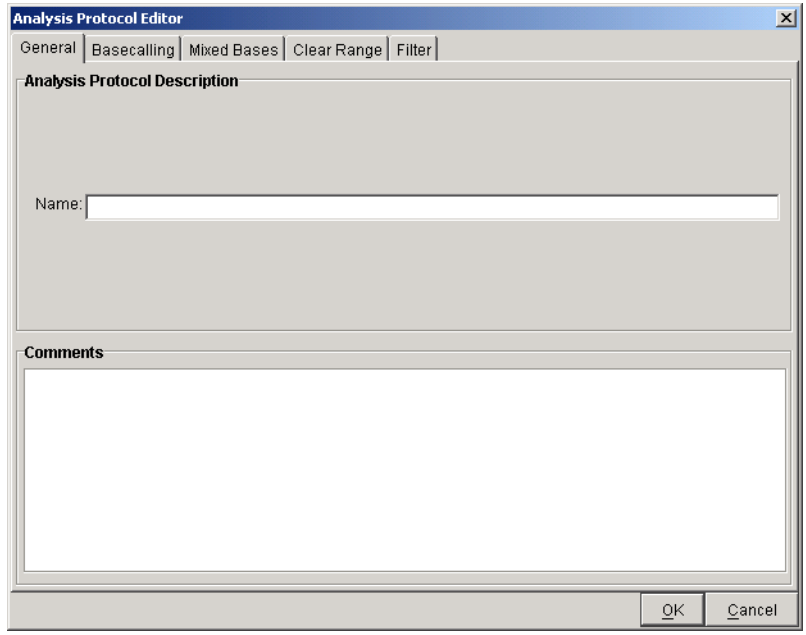


If more than one analysis application is installed on the data collection computer, the Analysis Applications dialog box opens.



2. Select **SeqScape**, then click **OK**.

   The Analysis Protocol Editor dialog box opens.



3. In the General tab, enter a unique name and description for the new protocol.

4. Select the **Basecalling** tab, then:
   a. Select the appropriate basecaller file as indicated below.

Basecaller	Description
KB.bcp	Algorithm calculates mixed or pure bases and sample quality values.
• Basecaller-3730POP7LR.bcp • Basecaller-3730POP7SR.bcp • Basecaller-3730POP7RR.bcp	Algorithm used in ABI PRISM Sequencing Analysis software v3.7.

   b. Select the appropriate Dye Set/Primer file as indicated below.

Basecaller	Dye Set/Primer File
KB.bcp	KB_3730_POP7_BDTv3.mob, or KB_3730_POP7_BDTv1.mob
• Basecaller-3730POP7LR.bcp • Basecaller-3730POP7SR.bcp • Basecaller-3730POP7RR.bcp	DT3730POP7{BDv3}.mob, or DT3730POP7{BD}.mob

IMPORTANT! Make sure that the basecaller and the DyeSet/Primer file types match.

c. If desired, select one or more stop points for data analysis. Base your selection on the basecaller being used as indicated below.

Option	Basecaller
At PCR Stop check box	KB or ABI
After __ Ns in __ bases check box	ABI
After __ Ns check box	ABI
After __ Bases check box	KB or ABI

5. Select the **Mixed Bases** tab, then:

   Note: This function is active with the KB basecaller only.

   a. For mixed bases only, select **Use Mixed Base Identification**.

   b. Use the default setting of 25% or change the detection level by entering a new value or dragging the % line up or down.

6. Select the **Clear Range** tab, then, if desired, select one or more stop points for data analysis.

   Note: The clear range is the region of the sequence that remains after excluding the low-quality or error-prone sequence at both the 5′ and 3′ ends.

7. Select the **Filter** tab, then, if desired, change one or more of the settings.

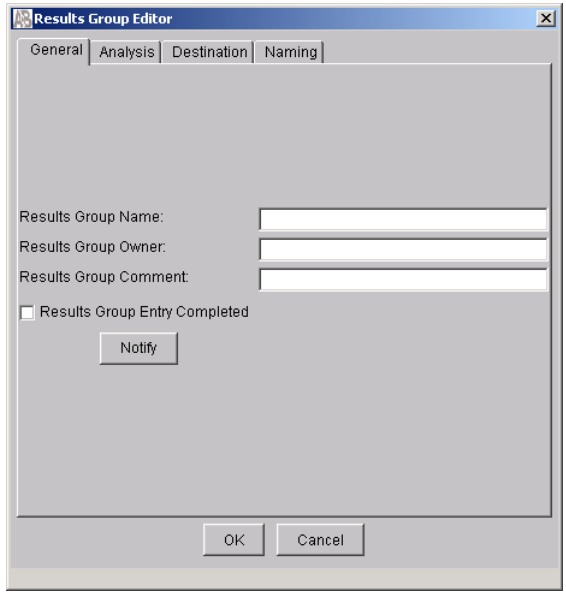
8. Select **OK** to save the protocol and close the Analysis Protocol Editor.

Creating a Results Group

To create a results group:

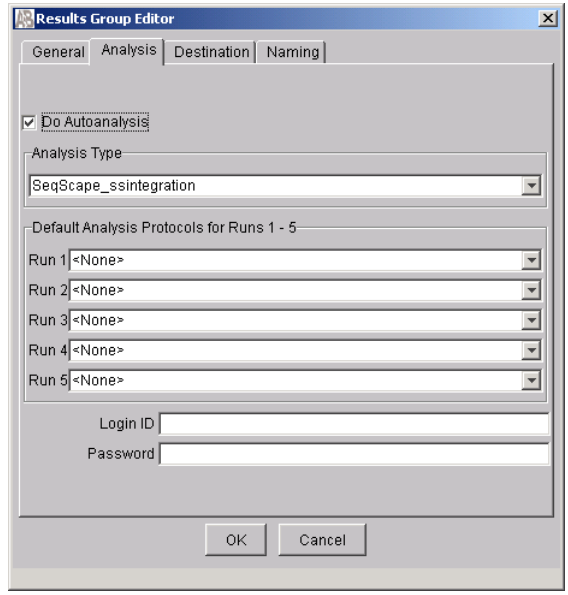
1. Click the **Results Group** icon in the navigation pane.

   The Results Group Editor opens.



2. Click **New**, or select an existing group, then click **Edit**.
3. In the General tab:
   a. Type a Results Group Name. The name can be used in naming and sorting sample files and it must be unique (see "File-Naming Convention" on page 2-9 for a list of accepted characters).
   b. Type a Results Group Owner. The owner name can be used in naming and sorting sample files.
   c. Type a Results Group Comment (optional).

4. Select the **Analysis** tab, then:



a. Select **Do Autoanalysis**.

b. Select **SeqScape_*YourInstrumentName*** in the Analysis Type drop-down list.

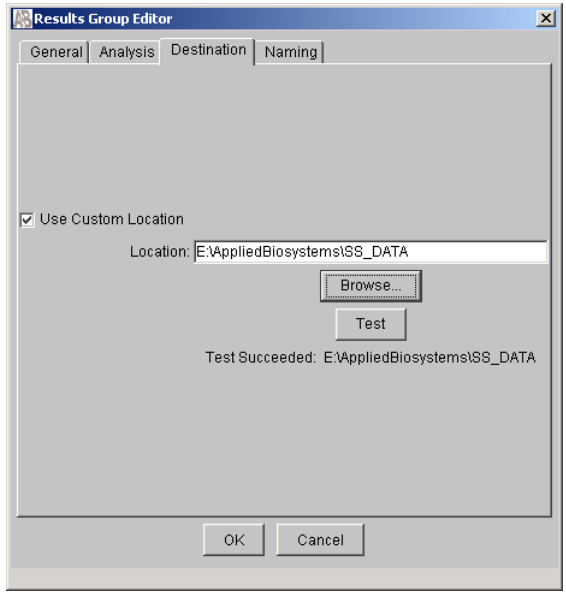
c. You can select default analysis protocols from the drop-down lists in the Default Analysis Protocols Runs 1–5 section. If you do not make selections here, you can manually select the analysis protocols when filling out the plate record.

Note: After you select a results group in a plate record, the Analysis Protocols are automatically filled in the plate record according to the defaults chosen here. A set of 96 samples can be run up to five consecutive times within the same plate record.

d. Type a valid SeqScape Login ID and Password in the text boxes.

IMPORTANT! Failure to use the proper login and password causes your samples not to be analyzed automatically.

5. Select the **Destination** tab, then:



a. Click **Use Custom Location**, then click **Browse** to navigate to a different save location.

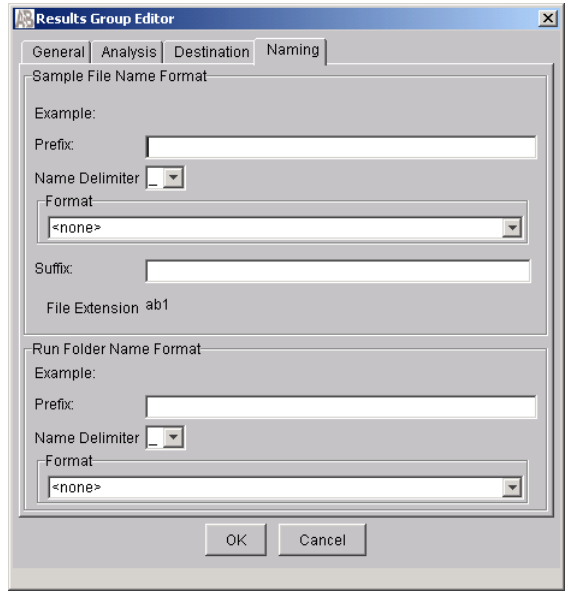
b. Click **Test** to test the Location path name connection:

   If it passes, a message box displays "Path Name test successful."

   If it fails, a message box displays "Could not make the connection. Please check that the Path Name is correct." Click and retry to establish a connection.

c. Click **OK**.

6. Select the **Naming** tab, then define custom names for sample file and run folder name, if desired.

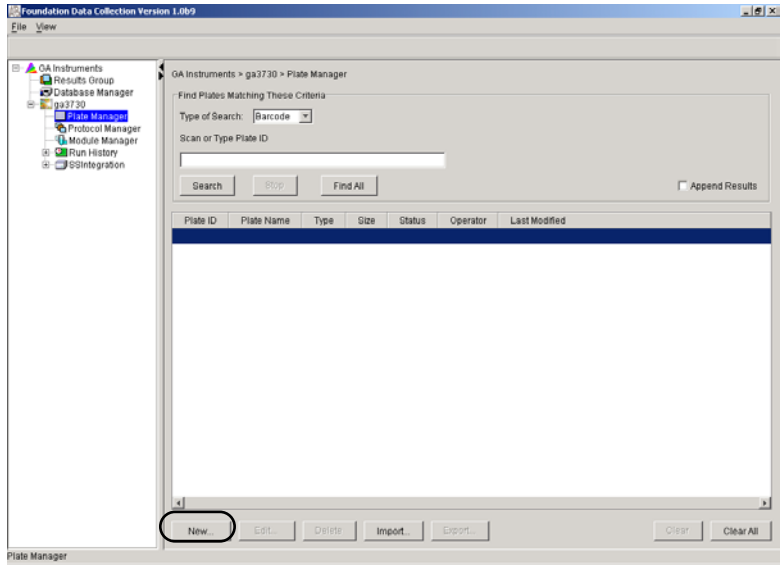


7. Click **OK** to close the Results Group Editor.

Creating a Plate Record

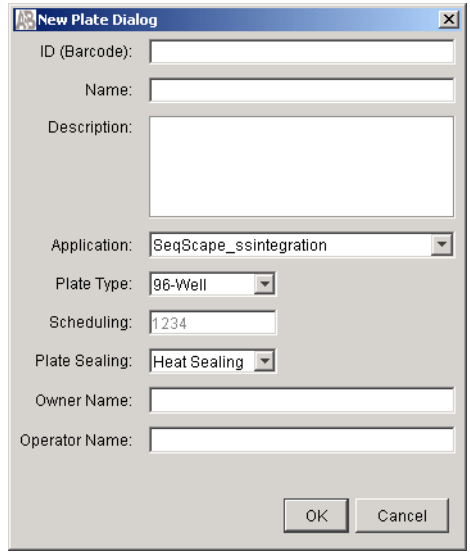
To create a new plate record:

1. Click the **Plate Manager** icon in the navigation pane.



2. Click **New**.

   The New Plate Dialog dialog box opens.



3. Complete the information in the New Plate Dialog:
   a. Type an ID for the plate.
   b. Type a name for the plate.
   c. Type a description for the plate (optional).
   d. Select **SeqScape_*YourInstrumentName*** in the Application drop-down list.
   e. Select **96-well** or **384-well** in the Plate Type drop-down list.
   f. For a 384-well plate, define a scheduling pattern.
   g. Select **Heat Sealing** or **Septa** in the Plate Sealing drop-down list.
   h. Type a name for the owner and operator.
   i. Click **OK**.

      The SeqScape Plate Editor opens (see Figure 11-3 on page 11-18).

Completing a Plate Record

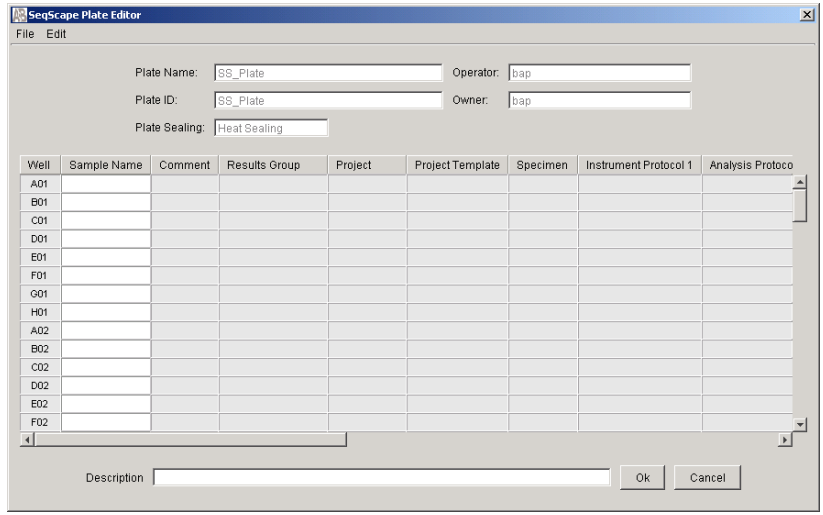


Figure 11-3 Blank Plate Editor

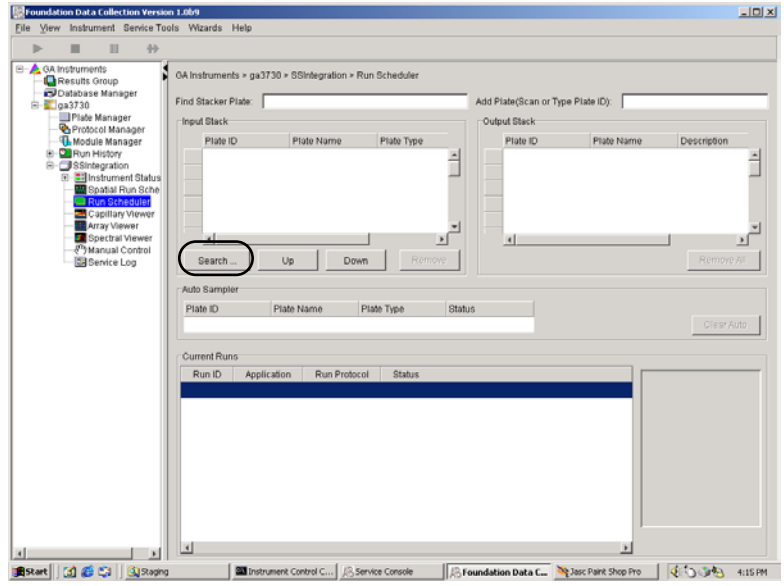
To complete a plate record:

1. Type sample names.
2. Select a results group or create a new one.
3. Select a project or create a new one. Based on the Project you select, the project template is filled in automatically.
4. Select a specimen or create one.
5. Select an instrument protocol or create one.
6. Select an analysis protocol or create one.
7. Click **OK**.

Scheduling and Starting a Run

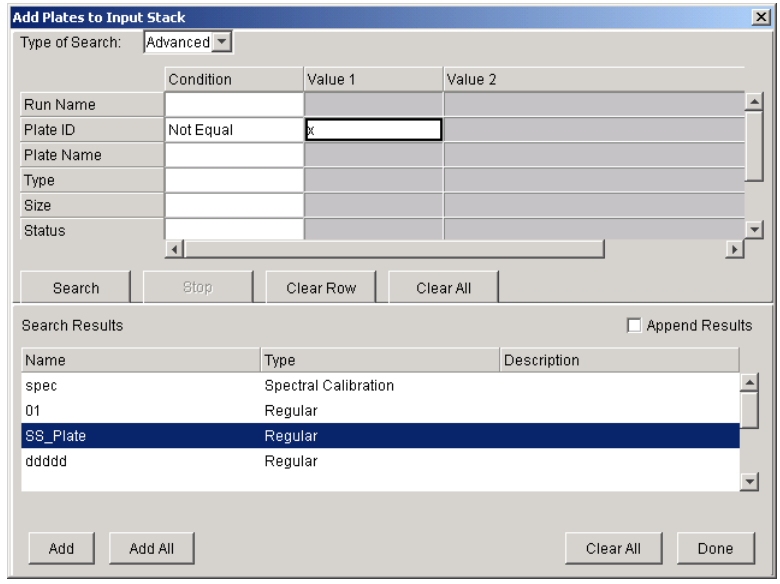
To schedule and then start a run:

1. Click the **Run Scheduler** icon in the navigation pane.



2. Click **Search** in the Input Rack section.

   The Add Plates to Input Stack dialog box opens.



3. Complete the Add Plates to Input Stack dialog box:
   a. Select **Advanced** in the Type of Search drop-down list.
   b. Enter your search criteria for your plate, then click **Search**.
   c. Select the plate in the Search Results section, then click **Add**.
   d. Click **Done**.

      The plate is displayed in the current Runs section of the Run Scheduler window.

4. Click  (Run) to start the run.

Autoanalysis Manager

Overview

Autoanalysis occurs in the following sequence:

- When data collection software finishes a run, the Message Service sends the message “Run Completed.”
- The Autoanalysis Manager receives the message, and the job is submitted. The job appears in the General tab.
- The Autoanalysis Manager polls for jobs every 2 minutes and opens the automated processing SeqScape version to analyze the data in the projects.
- At the end of analysis, the automated processing SeqScape version closes, and the status in the Autoanalysis Manager is updated.

Files Created

The data collection software stores the sample files in the location specified in the results group. The Autoanalysis Manager copies the files into the DataStore for SeqScape processing.

To maintain sufficient storage space on your hard drive, delete the sample files created by data collection software that are no longer needed.

Components

The Autoanalysis Manager has two tabs:

- General tab
- SeqScape tab

General Tab

The General tab shows the jobs that have been submitted and their status.

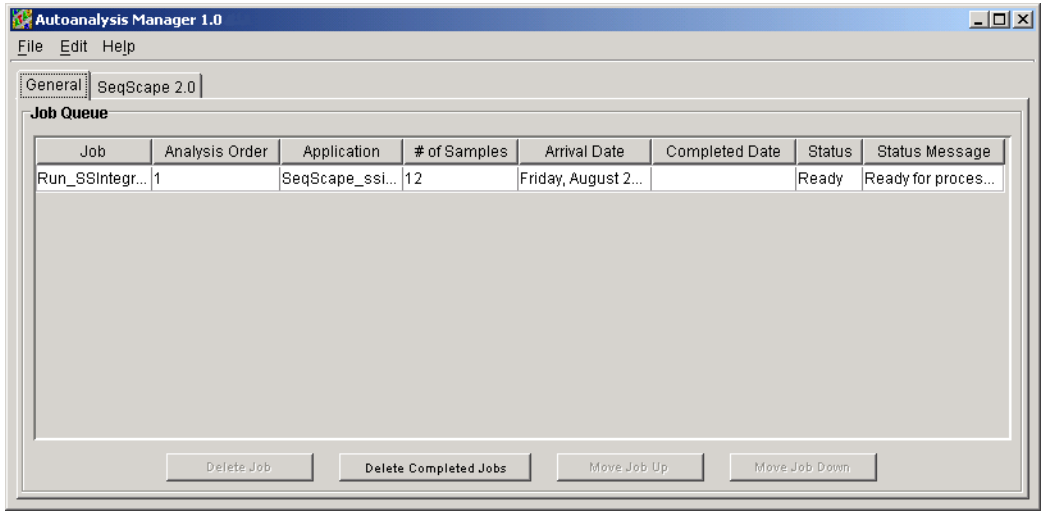


Figure 11-4 General Tab

Table 11-2 describes the functions of the command buttons in the General tab.

Table 11-2 General Tab Command Buttons

Button Name	Function
Delete Job	Deletes a pending job
Delete Completed Jobs	Deletes a completed job
Move Job Up	Moves a pending job higher in the queue
Move Job Down	Moves a pending job lower in the queue

SeqScape 2.0 Tab

The SeqScape 2.0 tab shows the jobs, project, and status information.

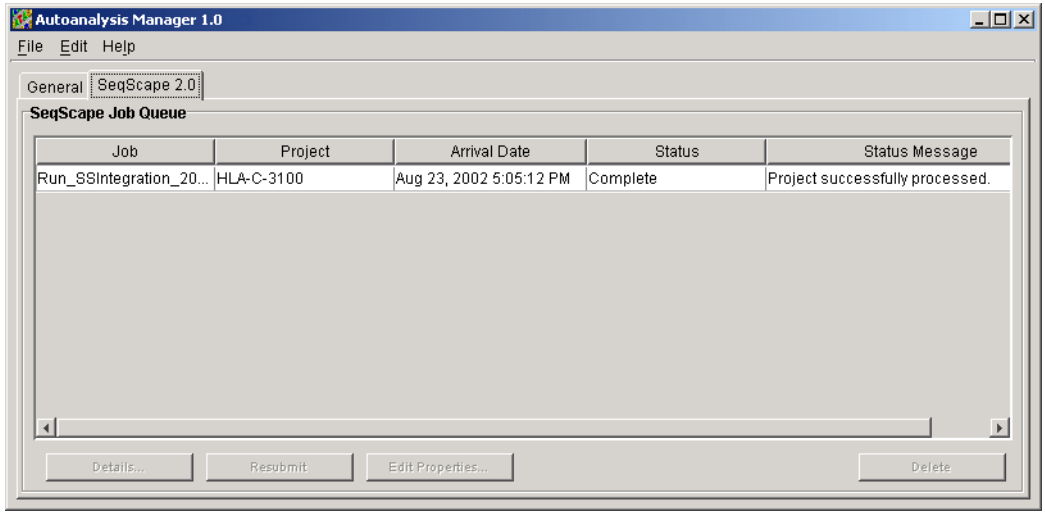
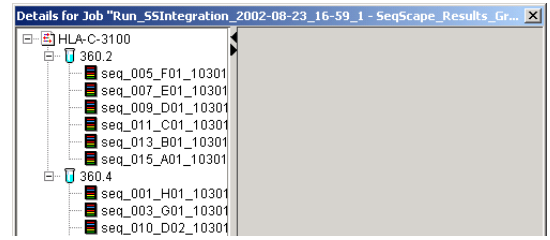


Figure 11-5 SeqScape 2.0 tab

Table 11-3 describes the functions of the command buttons in the SeqScape 2.0 tab:

Table 11-3 SeqScape 2.0 Tab Command Buttons

Button Name	Function
Details	Displays the project in the navigation pane 
Resubmit	Submits a job for analysis
Edit Properties	Edits the name and password (active only if analysis failed)
Delete	Deletes a job from the Autoanalysis Manager

File Sharing Between Data Collection and SeqScape Software

In Table 11-4, the term “files” refers to projects, project templates, specimens, and analysis protocols.

Table 11-4 File Sharing Table

Conditions	Result	Corrective Action
SeqScape software installed while data collection software was open (proper installation)		
• Files created in SeqScape • Data collection software open	Files are registered in both applications and are available for use in the data collection software.	—
• Files created in data collection software • SeqScape open	Files are registered in both applications and are available for use in the SeqScape software.	—
SeqScape software installed while data collection software was closed (improper installation)		
• Files created in SeqScape or in data collection software • Other software open or closed	SeqScape was never registered in the Data Service—no communication between the software.	1. Uninstall the SeqScape software. 2. Open the data collection software. 3. Reinstall the SeqScape software. 4. Register the software and define user IDs.

Basecallers and DyeSet/Primer Files

A

This appendix contains:

Definitions and Naming

Basecaller

A basecaller is an algorithm that determines the bases within a sequence during analysis. There are two types of basecallers:

- KB basecaller – A new algorithm that calculates mixed or pure bases, and sample quality values.
- ABI basecaller – An algorithm used in earlier versions of ABI PRISM® Sequencing Analysis and ABI PRISM® SeqScape® Software.

DyeSet/Primer

The DyeSet/Primer file compensates for the mobility differences between the dyes and primers and corrects the color code changes due to the type of chemistry used to label the DNA. DyeSet/Primer files are sometimes referred to as mobility files.

DyeSet/Primer File-Naming Conventions

DyeSet/Primer files use the following name convention:

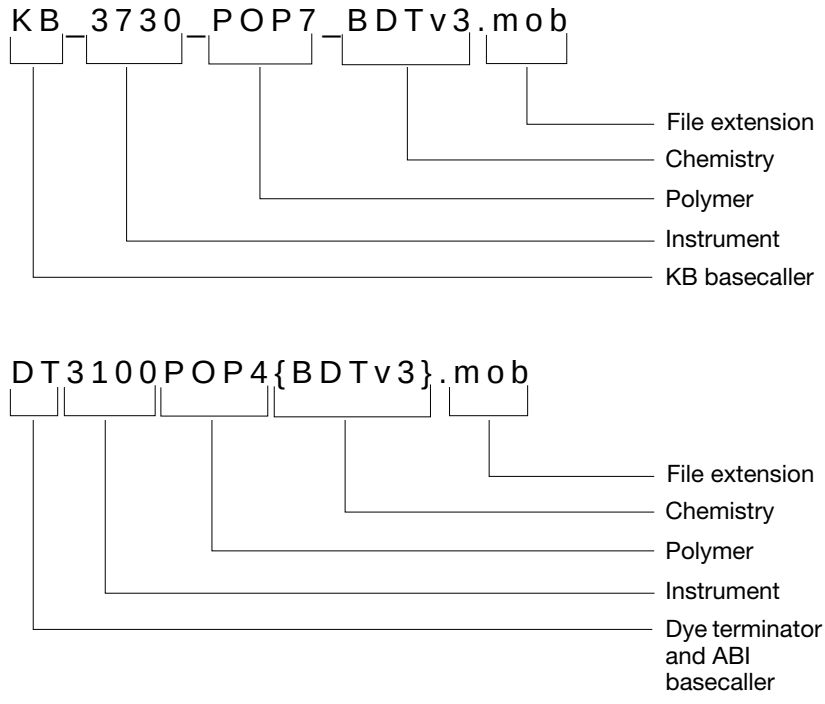


Figure A-1 Examples of DyeSet/Primer File Naming Convention

The DyeSet/Primer file names use a combination of characters to indicate the basecaller, instrument, chemistry, and polymer type as described in Table A-1.

Table A-1 DyeSet/Primer File Names

Abbreviation	For Runs Using …
Basecaller	
KB	KB basecaller
DP	Dye primer chemistry and the ABI basecaller
DT	Dye terminator chemistry, and the ABI basecaller
Type of Polymer or Gel	
4%Ac, 6%AC	% Acrylamide in the gel (377 instrument only)
5%LR	% Long Ranger in the gel (377 instrument only)
POP4	ABI PRISM® POP-4™ polymer
POP6	ABI PRISM® POP-6™ polymer
POP7	ABI PRISM® POP-7™ polymer
Chemistry	
BDTv3	ABI PRISM® BigDye® v3.0 and 3.1 Terminator chemistry
{BDv3}	
{BDv1}	ABI PRISM® BigDye® v1.0 and 1.1 Terminator chemistry
{BD}	
{-21M13}	Dye primer chemistry – the -21M13 primer is labeled
{M13Rev	Dye primer chemistry – the M13Rev primer is labeled

Basecaller and DyeSet/Primer Compatibility

The DyeSet/Primer file must match the chemistry and basecaller type that you are using

Note: DyeSet/Primer files are filtered based on the selected basecaller.

IMPORTANT! However, if you select the dyeSet/Primer file then select a basecaller file, no filtering of the basecaller list occurs. If you select a KB DyeSet/Primer file and an ABI basecaller for analysis, or a DT DyeSet/Primer file and an KB basecaller for analysis, the following error dialog box opens (see Figures A-2 and A-3).

Figure A-2 Error Message in the Sample Manager

Figure A-3 Error Message in the Analysis Protocol

ABI PRISM 310 Genetic Analyzer Files

Table A-2 Basecaller and DyeSet/Primer Files Used for Dye Terminator Chemistry

DNA Sequencing Chemistry	Capillary Array Length (cm)	Basecaller	DyeSet/Primer
ABI Basecalling			
ABI PRISM BigDye Terminator	47	310POP4	DT310POP4{BD}v2.mob
	47	310POP6	DT310POP6{BD}2.mob DTPOP6{BDSet-AnyPrimer}.mob
	61	310POP6	DT310POP6{BD}2.mob DTPOP6{BDSet-AnyPrimer}.mob
ABI PRISM dRhodamine Terminator	47	310POP4	DT310POP4{dRhod}v1.mob
	47	310POP6	DT310POP6{dRhod}v2.mob DTPOP6{dRhod-AnyPrimer}.mob
	61	310POP6	DT310POP6{dRhod}v2.mob DTPOP6{dRhod-AnyPrimer}.mob
ABI PRISM BigDye v3 Terminator	47	310POP4	DT310POP4{BDv3}v2.mob
	47	310POP6	DT310POP6{BDv3}v2.mob
	61	310POP6	DT310POP6{BDv3}v2.mob

Table A-3 Basecaller and DyeSet/Primer Files Used for Dye Primer Chemistry

DNA Sequencing Chemistry	Capillary Array Length (cm)	Basecaller	DyeSet/Primer
ABI Basecaller			
ABI PRISM BigDye Primer	47	310POP4	DP310POP4{BD-21M13}v1.mob DP310POP4{M13Rev}v1.mob
	47	310POP6	DP310POP6{BD-21M13}v1.mob DP310POP6{M13Rev}v1.mob
	61	310POP6	DP310POP6{BD-21M13}v1.mob DP310POP6{M13Rev}v1.mob
ABI PRISM BigDye v3 Primer	47	310POP4	DP310POP4{BDv3-21M13}v1.mob DP310POP4{BDv3-M13Rev}v1.mob
	47	310POP6	DP310POP6{BDv3-21M13}v1.mob DP310POP6{BDv3-M13Rev}v1.mob
	61	310POP6	DP310POP6{BDv3-21M13}v1.mob DP310POP6{BDv3-M13Rev}v1.mob

ABI PRISM 377 DNA Sequencer Files

Table A-4 Basecaller and DyeSet/Primer Files Used for Dye Terminator Chemistry

DNA Sequencing Chemistry	WTR (cm)/Scan Rate (scans/hr)	Basecaller	DyeSet/Primer
ABI Basecalling			
• ABI PRISM Dye Terminator	36/2400	Basecaller-377.bcp	DT3774%Ac{A Set-Any Primer}.mob
	36 & 48/1200	Basecaller-377LR.bcp	
• ABI PRISM BigDye Terminator • ABI PRISM dGTP BigDye Terminator	36/2400	Basecaller-377.bcp	DT377{BD}.mob
	36 & 48/1200	Basecaller-377LR.bcp	
ABI PRISM dRhodamine Terminator	36/2400	Basecaller-377.bcp	DT377{dR Set-Any Primer}.mob
	36 & 48/1200	Basecaller-377LR.bcp	
• ABI PRISM BigDye v3 Terminator	36/2400	Basecaller-377.bcp	DT377{BDv3}v1.mob
• ABI PRISM dGTP BigDye v3.0Terminator	36 & 48/1200	Basecaller-377LR.bcp	DT377LR{BDv3}v2.mob

Table A-5 Basecaller and DyeSet/Primer Files Used for Dye Primer Chemistry

DNA Sequencing Chemistry	WTR (cm)	Basecaller	DyeSet/Primer
ABI Basecalling			
ABI PRISM BigDye Primer	36/2400	Basecaller-377.bcp	DP377-5%LR{BD-21M13}.mob, or DP377-5%LR{BD-M13Rev}.mob,
	36 & 48/1200	Basecaller-377LR.bcp	
ABI PRISM BigDye v3 Primer	36/2400	Basecaller-377.bcp	DP377{BDv3-21M13}v1.mob, or DP377{BDv3-M13Rev}v1.mob
	36 & 48/1200	Basecaller-377LR.bcp	
ABI PRISM Dye Primer	36/2400	Basecaller-377.bcp	DP377-4%Acv2{M13Rev}.mob, DP377-4%Acv2{-21M13}.mob, DP377-4%Acv2{KS}.mob, DP377-4%Acv2{SK}.mob, DP377-4%Acv2{SP6}.mob, DP377-4%Acv2{T3}.mob, DP377-4%Acv2{T7}.mob, DP377-6%Acv2{M13Rev}.mob, DP377-6%Acv2{-21M13}.mob, DP377-6%Acv2{SK}.mob, DP377-6%Acv2{SP6}.mob, DP377-6%Acv2{T3}.mob, or DP377-6%Acv2{T7}.mob
	36 & 48/1200	Basecaller-377LR.bcp	

ABI PRISM 3100 Genetic Analyzer Files

Table A-6 Basecaller and DyeSet/Primer Files Used for Dye Terminator Chemistry

DNA Sequencing Chemistry	Capillary Array Length (cm)	Basecaller	DyeSet/Primer
KB Basecalling			
ABI PRISM BigDye v3.0 Terminator	50: std read	KB.bcp	KB_3100_POP6_BDTv3_SR.mob
ABI PRISM BigDye Terminator			KB_3100_POP6_BDTv1_SR.mob
ABI Basecalling			
• ABI PRISM BigDye v3.0 Terminator • ABI PRISM dGTP BigDye v3.0Terminator	36: ultra rapid	Basecaller-3100POP4UR.bcp	DT3100POP4{BDv3}v1.mob
	80: long read	Basecaller-3100POP4_80cmv3.bcp	
	36: rapid read	Basecaller-3100POP6RRv2.bcp	DT3100POP6{BDv3}v1.mob
	50: std read	Basecaller-3100POP6SR.bcp	
• ABI PRISM BigDye Terminator • ABI PRISM dGTP BigDye Terminator	36: ultra rapid	Basecaller-3100POP4UR.bcp	DT3100POP4LR{BD}v1.mob
	80: long read	Basecaller-3100POP4_80cmv3.bcp	
	36: rapid read	Basecaller-3100POP6RRv2.bcp	DT3100POP6{BD}v2.mob
	50: std read	Basecaller-3100POP6SR.bcp	

Table A-6 Basecaller and DyeSet/Primer Files Used for Dye Terminator Chemistry *(continued)*

DNA Sequencing Chemistry	Capillary Array Length (cm)	Basecaller	DyeSet/Primer
ABI PRISM dRhodamine Terminator	36: ultra rapid	Basecaller-3100POP4UR.bcp	DT3100POP4{dRhod}v2.mob
	80: long read	Basecaller-3100POP4_80cmv3.bcp	
	36: rapid read	Basecaller-3100POP6RRv2.bcp	DT3100POP6{dRhod}v2.mob*
	50: std read	Basecaller-3100POP6SR.bcp	

*If ABI PRISM® Sequencing Analysis Software v3.7 is on computer, then two versions of the DT3100POP6{dRhod} mobility file exist. Use the newest version, DT3100POP6{dRhod}v2.mob, instead of DT3100POP6{dRhod}v1.mob.

Table A-7 Basecaller and DyeSet/Primer Files Used for Dye Primer Chemistry

DNA Sequencing Chemistry	Capillary Array Length (cm)	Basecaller	DyeSet/Primer
ABI Basecalling			
ABI PRISM BigDye Primer	36: rapid read	Basecaller-3100POP6RRv2.bcp	DP3100POP6{BD-21M13}v1.mob DP3100POP6{BD-M13Rev}v1.mob
	50: std read	Basecaller-3100POP6SR.bcp	
ABI PRISM BigDye v3 Primer	36: rapid read	Basecaller-3100POP6RRv2.bcp	DP3100POP6{BDv3-21M13}v1.mob DP3100POP6{BDv3-M13Rev}v1.mob
	50: std read	Basecaller-3100POP6SR.bcp	
ABI PRISM BigDye v3 Primer (All primers)	36: ultra rapid	Basecaller-3100POP4UR.bcp	DP3100POP4{BDv3}v1.mob
	80: long read	Basecaller-3100POP4_80cmv3.bcp	

ABI PRISM 3100-*Avant* Genetic Analyzer Files

Table A-8 Basecaller and DyeSet/Primer Files Used for Dye Terminator Chemistry

DNA Sequencing Chemistry	Capillary Array Length (cm)	Basecaller	DyeSet/Primer
KB Basecalling			
ABI PRISM BigDye v3.0 Terminator	50: std read	KB.bcp	KB_3100_POP6_BDTv3_SR.mob
ABI PRISM BigDye Terminator			KB_3100_POP6_BDTv1_SR.mob
ABI Basecalling			
ABI PRISM BigDye v3.0 Terminator	36: ultra rapid	Basecaller-3100APOP4UR.bcp	DT3100POP4{BDv3}v1.mob
	80: long read	Basecaller-3100APOP4_80cmv3.bcp	
	36: rapid read	Basecaller-3100APOP6RRv2.bcp	DT3100POP6{BDv3}v1.mob
ABI PRISM BigDye Terminator	36: ultra rapid	Basecaller-3100APOP4UR.bcp	DT3100POP4LR{BD}v1.mob
	80: long read	Basecaller-3100APOP4_80cmv3.bcp	
	36: rapid read	Basecaller-3100APOP6RRv2.bcp	DT3100POP6{BD}v2.mob
	50: std run	Basecaller-3100APOP6SR.bcp	

Table A-8 Basecaller and DyeSet/Primer Files Used for Dye Terminator Chemistry *(continued)*

DNA Sequencing Chemistry	Capillary Array Length (cm)	Basecaller	DyeSet/Primer
ABI PRISM dRhodamine Terminator	36: ultra rapid	Basecaller-3100APOP4UR.bcp	DT3100POP4{dRhod}v2.mob
	80: long read	Basecaller-3100APOP4_80cMV3.bcp	
	36: rapid read	Basecaller-3100APOP6RRv2.bcp	DT3100POP6{dRhod}v2.mob*
	50: std run	Basecaller-3100APOP6SR.bcp	

*If Sequencing Analysis software is on the computer, then two versions of the DT3100POP6{dRhod} mobility file exist. Use the newest version, DT3100POP6{dRhod}v2.mob, instead of DT3100POP6{dRhod}v1.mob.

ABI PRISM 3700 DNA Analyzer Files

Table A-9 Basecaller and DyeSet/Primer Files Used for Dye Terminator Chemistry

DNA Sequencing Chemistry	Capillary Array Length (cm)	Basecaller	DyeSet/Primer
ABI Basecalling			
ABI PRISM BigDye v3.0 Terminator	50	Basecaller-3700POP6.bcp	DT3700POP6{BDv3}v1.mob
		Basecaller-3700POP5LR.bcp	DT3700POP5{BDv3}v1.mob
ABI PRISM BigDye Terminator	50	Basecaller-3700POP6.bcp	DT3700POP6{BD}v5.mob
		Basecaller-3700POP5LR.bcp	DT3700POP5{BD}v3.mob
ABI PRISM dRhodamine Terminator	50	Basecaller-3700POP6.bcp	DT3700POP6{dRhod}v3.mob
		Basecaller-3700POP5LR.bcp	DT3700POP5{dRhod}v1.mob

Table A-10 Basecaller and DyeSet/Primer Files Used for Dye Primer Chemistry

DNA Sequencing Chemistry	Capillary Array Length (cm)	Basecaller	DyeSet/Primer
ABI Basecalling			
ABI PRISM BigDye v3 Primer	50	Basecaller-3700POP6.bcp	DP3700POP6{BDv3-21M13}v1.mob
			DP3700POP6{BDv3-M13Rev}v1.mob
		Basecaller-3700POP5LR.bcp	DP3700POP5{BDv3-21M13}v1.mob
			DP3700POP5{BDv3-M13Rev}v1.mob
ABI PRISM BigDye Primer	50	Basecaller-3700POP6.bcp	DP3700POP6{BD-21M13}v3.mob
			DP3700POP6{BD-M13Rev}v2.mob
		Basecaller-3700POP5LR.bcp	DP3700POP5{BD-21M13}v1.mob
			DP3700POP5{BD-M13Rev}v1.mob

Applied Biosystems 3730/3730*xl* DNA Analyzers Files

Table A-11 Basecaller and DyeSet/Primer Files Used for Dye Terminator Chemistry

DNA Sequencing Chemistry	Capillary Array Length (cm)	Basecaller	DyeSet/Primer
KB Basecalling			
ABI PRISM BigDye v3.0 Terminator	all lengths	KB.bcp	KB_3730_POP7_BDTv3.mob
ABI PRISM BigDye Terminator	all lengths	KB.bcp	KB_3730_POP7_BDTv1.mob
ABI Basecalling			
ABI PRISM BigDye v3.0 Terminator	36: rapid read	Basecaller-3730POP7RR.bcp	DT3730POP7{BDv3}.mob
	36: std read	Basecaller-3730POP7SR.bcp	
	50: long read	Basecaller-3730POP7LR.bcp	
ABI PRISM BigDye Terminator	36: rapid read	Basecaller-3730POP7RR.bcp	DT3730POP7{BD}.mob
	36: std read	Basecaller-3730POP7SR.bcp	
	50: long read	Basecaller-3730POP7LR.bcp	

Frequently Asked Questions B

This appendix contains:

General Questions and Answers

This appendix provides answers to the most commonly asked questions regarding the ABI PRISM® SeqScape® v2.0 Software.

Table B-1 General Questions

Question	Answer
How does SeqScape software v2.0 differ from v1.1?	Refer to Chapter 1, "Introduction to ABI Prism SeqScape Software."
What ABI instruments can I use to generate data for SeqScape software?	Seqscape software analyzes sequence files generated from ABI PRISM 3700, 3100, 377, 310, 3730, and 3730*xl* genetic analyzers. The software also accepts text sequences in FASTA format.
What ABI chemistries are supported?	• ABI PRISM® BigDye® Terminator v3.1, v3, v2, v1.1, and v1 chemistries • ABI PRISM® BigDye® Primers and dRhodamine dyes
What are the computer requirements for SeqScape software?	• CPU – 733 MHz or faster, single processor • Memory – 256 MB RAM • OS – Windows NT® 4 or Windows® 2000 with Service Pack 1 or 2 • 1 GB hard drive • Pentium® III or IV chip, *not* Xenon
What kind of performance can I expect from my SeqScape software?	That depends on your computer specifications. For example, if you have a computer with an 850 MHz processor, 256 MB RAM, Pentium III chip, running on Windows 2000 and are analyzing 100 samples files, 10 specimens, and a 1Kb reference, the analysis time is 2 min.
Does SeqScape software support BioLIMS/Sequence Collector?	Yes, SeqScape software can connect to Sequence Collector v3.0 databases and read sample files. Those sample files can be added into a SeqScape software project and analyzed. The results will be saved back to the database.

Table B-1 General Questions *(continued)*

Question	Answer
How does SeqScape software compare to MicroSeq® and ViroSeq™ software?	SeqScape software – compares samples to a reference sequence MicroSeq software – identifies bacteria ViroSeq software – identifies genotype HIV-1 resistance mutations
Do I need Sequencing Analysis software if I have SeqScape software?	Sequencing Analysis software is a multi-purpose software used to analyze, edit, view, display, and print sequencing sample files. SeqScape software is designed specifically for resequencing. Sequencing Analysis software should be used in every laboratory for general troubleshooting and viewing of data.
How can I share my work with someone at a different site? What should I send them?	All sample files, analysis parameters, reference sequence, and analysis results are saved in every SeqScape project file. These files can be shared with anyone who has the software. You can also share project templates, which contain the reference sequence and analysis parameters. A colleague can then analyze sample files of their choice using the project templates to create a new project. The analysis is identical to your own analysis with the same project template.
Can I BLAST against a database?	To search a database using a sequence generated with SeqScape software, in the Project view, export the NT alignment as an aligned FASTA file by selecting **File** > **Export**. Open this file in a text viewer, then cut and paste the sequence you would like to search for in your BLAST query. Refer to Chapter 9, “Exporting and Printing Data and Reports,” for detailed information on exporting.
Can I put samples from different individuals in the same specimen?	No, each individual sample should be in a different specimen. Refer to chap 6

Table B-1 General Questions *(continued)*

Question	Answer
What alignment algorithms are used in SeqScape software?	The sample assembly and specimen alignments are generated using a Smith-Waterman local sequence alignment algorithm using parameters appropriate for DNA sequencing.
Can SeqScape software perform just the alignment for samples?	No. Samples must be basecalled within SeqScape software to take advantage of the assembly and resequencing algorithms.
What can I print in SeqScape software?	Views only for sample, specimen, and project and complete reports.
What printers are recommended for use with SeqScape software?	An HP® 8100, 4500, or an Epson® 900 color printer is recommended.

SeqScape Manager Questions and Answers

Table B-2 SeqScape Manager Questions

Question	Answer
What is the SeqScape Manager?	SeqScape Manager allows you to import, export, create, and delete projects, project templates, reference data groups, analysis defaults, and display settings. Access SeqScape Manager by selecting it from the Tools menu.
What is an object:	An object is a named collection of data elements to perform certain functions, for example, analysis protocol.
How do I create a new user?	You must be logged in as Admin user. 1. Select **Tools** > **Options**. 2. Select the **Users** tab, then click **New**. 3. Enter the new user name (be sure to omit any spaces in the user name), then click **OK**. 4. To log in with the new name, exit the software, then relaunch it. 5. Log in with the new user name.
What is a project in SeqScape software?	A project is created using a project template. Projects contain sample data files grouped into specimens.
What is a project template?	A project template is the mold from which projects are created. Templates contain: analysis defaults, display settings, and a reference data group (RDG).
What is a specimen?	A specimen contains all the sample data from a single biological source.
Can I mix samples from different biological sources?	It is not possible to analyze data from different biological sources in the same specimen.

Table B-2 SeqScape Manager Questions *(continued)*

Question	Answer
What is a reference data group (RDG)?	The RDG is an essential part of the project template that contains all of the analysis-specific information, including the reference sequence, translation codon table, known variants, RDG name, reference segments, regions of interest (ROI), layers, and the name of the associated allele libraries.
What is a reference sequence?	A reference sequence is the backbone sequence against which the software compares the consensus segments. A reference sequence contains continuous or discontinuous sequences made up of one or more reference segments
What is a reference segment?	A reference segment is a contiguous segment within the reference sequence that corresponds to a single contiguous DNA sequence.
What is a reference break?	A reference break is a break in the reference sequence between two reference segments where the reference is not contiguous.
What is a translation codon table?	A table that translates amino acid and genetic codes. Refer to Appendix C, “Translation Tables.”
What is a known variant?	An AA variant or NT variant that has been previously identified in the reference.
What is a region of interest (ROI)	An ROI is a region on the reference segment with special numbering properties used for display.
How can I configure a reference segment and ROIs within it?	After you import a reference sequence into the RDG, use the ROI tab to reconfigure a reference segment and to add ROIs.
What if I do not have variant information?	Variants are not necessary to create a reference data group. If you do import variants, they must be in a tab-delimited text file format or FASTA alignment of sequences.

Table B-2 SeqScape Manager Questions *(continued)*

Question	Answer
What kinds of files can I import into SeqScape software?	ABI sample files, tab-delimited text, and FASTA file format can be imported into the software.
Can analyzed data be used in SeqScape software?	Analyzed data can be used. However, if it is in the ABI data format (and not FASTA), any prior analysis, results, and edits will be overwritten when the files are reanalyzed using SeqScape software.
What can I export from SeqScape software?	User information, projects, project alignments, project templates, reports, nucleotide and amino acid variants, and libraries can be exported from the software. Refer to Appendix D, “User Privileges.”
Can I export each consensus sequence individually?	Consensus sequences for a project can be exported as a group by using selecting **File** > **Export** in the Project view.
What is FASTA format? How can I convert non-FASTA files into the correct format?	A sequence in FASTA format begins with a single-line description, followed by lines of sequence data. The description line is distinguished from the sequence data by a greater-than (>) symbol in the first column. **Note:** When creating a file in Microsoft® Word, be sure to save it in text-only format (line breaks are OK, but spaces are not OK). >HumMitoCamb from 15871 to 450 (hard return) aatactcaaatgggcctgtccttgtagtataaactaataca ccagtcttgtaaaccggagatgaaaacctttccaaggac aaatcagagaaaaagtctttaactccaccattagcaccc aaagct (hard return)
What are Analysis Settings?	The analysis settings determine the basecalling, mixed base settings, clear range, and filter settings.
What is Clear Range?	Clear range defines the range of usable sample sequence to be included in the consensus.

Table B-2 SeqScape Manager Questions *(continued)*

Question	Answer
What are Filter Settings?	These allow you to set the maximum percentage of mixed-bases allowed, maximum Ns allowed, minimum clear range length, and the minimum sample score for each sample. Samples failing the filter checks will not be included in the analysis.
What are Display Settings?	These control the font styles and colors for bases, electropherogram display and axis scale, display view for variants, and display views for nucleotide translation.

Analysis and Reports Questions and Answers

Table B-3 Analysis and Reports Questions

Question	Answer
What does it mean when there is a red line across a specimen?	Strike through symbols indicate that analysis needs to be performed.
How do I begin analysis?	Click the green arrow button in the toolbar or select **Analysis** > **Analyze**.
Can the SeqScape software handle gaps in sequence?	SeqScape software automatically inserts gaps in the sample and consensus sequences if these gaps are necessary to produce clean sequence alignments. Gaps should be removed before importing sequences from FASTA-formatted files.
What does the Alignment Score mean in the Analysis Report?	The alignment score shows the number of characters that were inserted in each specimen consensus to create the project alignment. A lower alignment score indicates more similarity between the specimen consensus and the reference.
How does editing affect my data? What gets updated?	If you insert, delete, or change a base within a sample, the change is reflected in the consensus sequence. All samples change to reflect the consensus edits.
How can I distinguish between edited and non-edited data?	When a base is edited, it is displayed as lower-case while the unedited bases are displayed in upper-case letters.
What will happen to my edited sequence when I start analysis?	Once basecalling begins, all current edits are overwritten. Changes to the analysis settings that do not require re-basecalling of the sample preserve edits and the reference sequence.
What happens if I edit a consensus base?	The base changes to lowercase in the consensus and the quality bar turns gray. All bases in the samples at that position that disagreed with the new basecall are changed to agree with the new consensus base and are shown in lowercase with a gray quality bar.

Table B-3 Analysis and Reports Questions *(continued)*

Question	Answer
How do I remove unwanted spaces in my samples?	To remove unwanted spaces in the sample, double-click the space and press the Delete key.
What can I do if I deleted too many bases?	Repeat the analysis.
How can I access my various reports?	Access all reports by clicking the corresponding button in the toolbar or by selecting the desired report from the Analysis menu.
What is the Nucleotide Variant Report?	This report displays all the positions of variance from the reference, known and unknown, for each specimen in the project.
What is the Amino Acid Variant Report?	This report displays the location of the known and unknown amino acid variants.
What is the Analysis Comparison Report?	This report summarizes all the nucleotide variants in the project.
What do the percentages mean in the Analysis Comparison Report?	These indicate the percent of specimens in which a particular nucleotide occurs in this position.
What is the Specimen Report?	This report summarizes all of the data generated for each specimen in the project.
How can I edit my specimen name?	Select the specimen and select **Edit** > **Rename**.
How can I delete samples or specimens?	Select the item to be deleted, then select **Edit** > **Delete**, Click the Delete button on the toolbar, or press the Delete key on the keyboard.
What is the TraceTuner™ basecaller module?	The TraceTuner basecalling module in SeqScape software is responsible for generating per-base sample quality values and identifying mixed bases.
What are quality values?	A quality value is an estimation of the certainty for a basecall in the sample (sample QV) or consensus (consensus QV).

Table B-3 Analysis and Reports Questions *(continued)*

Question	Answer
How is the Basecaller Quality Value generated?	It is derived using an algorithm that is designed to examine the certainty of basecalls. See Chapter 10, “Sample and Consensus Quality Values,” for more information.
What is the Quality Value equation?	$QV = -10\log_{10}(PE)$ where PE is the probability of error.
How are Sample Quality Values generated?	They are generated using a statistical algorithm which is calibrated to estimate the certainty of basecalls.
How is a Sample Quality Value different from the Sample Score?	The sample score is the average quality value of the bases in the clear range sequence for that sample. A sample quality value is a per-base estimate of basecaller accuracy.
How does the Consensus Quality Value differ from the Consensus Score?	The consensus score is the average quality value of the bases in the consensus sequence for that specimen. A consensus quality value is a per-base estimate of the accuracy of the consensus-calling algorithm.

Translation Tables

C

This appendix contains:

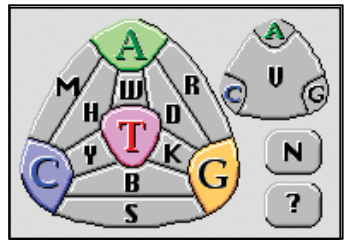
IUPAC/IUB Codes

Table C-1 IUPAC/IUB Codes

Code	Translation
A	Adenosine
C	Cytidine
G	Guanosine
T	Thymidine
B	C, G, or T
D	A, G, or T
H	A, C, or T
R	A or G (puRine)
Y	C or T (pYrimidine)
K	G or T (Keto)
M	A or C (aMino)
S	G or C (Strong—3 H bonds)
W	A or T (Weak—2 H bonds)
N	aNy base
V	A, C, or G

IUPAC Diagrams

IUPAC



IUPAC heterozygous

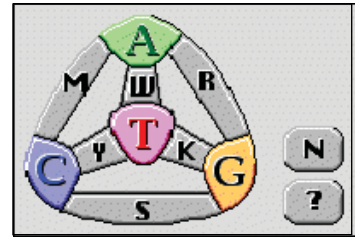


Figure C-1 IUPAC Diagrams

Complements

Table C-2 Complements

A	T	S	S
C	G	W	W
G	C		
T	A	B	V
		D	H
R	Y	H	D
Y	R	V	B
K	M	N	N
M	K		

Universal Genetic Code

Table C-3 Universal Genetic Codes

5' End	2nd Position				3' End
	T	C	A	G	
T	Phe	Ser	Tyr	Cys	T
	Phe	Ser	Tyr	Cys	C
	Leu	Ser	OCH	OPA	A
	Leu	Ser	AMB	Trp	G
C	Leu	Pro	His	Arg	T
	Leu	Pro	His	Arg	C
	Leu	Pro	Gln	Arg	A
	Leu	Pro	Gln	Arg	G
A	Ile	Thr	Asn	Ser	T
	Ile	Thr	Asn	Ser	C
	Ile	Thr	Lys	Arg	A
	Met	Thr	Lys	Arg	G
G	Val	Ala	Asp	Gly	T
	Val	Ala	Asp	Gly	C
	Val	Ala	Glu	Gly	A
	Val	Ala	Glu	Gly	G
Stop Codes: AMBer, OCHer, OPA					

Amino Acid Abbreviations

Table C-4 Amino Acid Abbreviations

Amino Acid	Three Letters	One Letter
Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartic Acid	Asp	D
Cysteine	Cys	C
Glutamic Acid	Glu	E
Glutamine	Gln	Q
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Threonine	Thr	T
Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V
Any Amino Acid		X

User Privileges

D

Tables of User Privileges

This appendix contains a list of privileges for users of the three categories, Administrator, Scientist, and Analyst, when they use the ABI PRISM® SeqScape® Software Version 2.0.

Table D-1 Access for Admin Level

		Description of access for users of Admin level only	Admin	Scientist	Analyst
Admin only access	1	Create User Accounts	Allowed	Not Allowed	Not Allowed
	2	Exporting/Importing User Accounts			
	3	Export a Project/PT/RDG/Library from the SeqScape Manager			
	4	Import objects from outside the DataStore into the SeqScape Manger			
	5	Install SeqScape for an automated analysis system			

Table D-2 Access for Admin and Scientist Levels

		Description of access for users of Admin and Scientist levels	Admin	Scientist	Analyst
SeqScape Manager	1	Delete an object from the SeqScape manager	Allowed	Allowed	Not Allowed
	2	Delete a Project from the SeqScape manager			
	3	Save As.. an object in the SeqScape manager			
	4	Create a new object in the SeqScape Manager			
	5	Create a new Project Template in SeqScape Manager			
	6	Configure analysis defaults in SeqScape manager			
	7	Deleting entries from a library in the SeqScape manager			
	8	Re-Configure an existing Project Template in the SeqScape Manager			
Analysis Protocol & Settings	9	Creating an analysis protocol	Allowed	Allowed	Not Allowed
	10	Editing an existing analysis protocol			
	11	Apply an analysis protocol to a set of samples (project/sample/specimen)			
	12	Create new Primary Seq Analysis Protocols			
	13	Set Clear range determination in Analysis settings or analysis defaults			
	14	Set Mixed Base determination in Analysis settings or analysis defaults in a Project, PT/SS Manager			

Table D-2 **Access for Admin and Scientist Levels** *(continued)*

		Description of access for users of Admin and Scientist levels	Admin	Scientist	Analyst
RDG	15	RDG: Import Variants and Reference into an RDG from a set of aligned FASTA files	Allowed	Allowed	Not Allowed
	16	RDG general tab: configure an RDG in general tab			
	17	RDG ROI tab: Edit a Reference Data Group (RDG): configure Layers			
	18	RDG ROI tab: Edit a Reference Data Group (RDG): configure ROIs			
	19	RDG ROI tab: Edit a Reference Data Group to use an implicit reference			
	20	RDG ROI tab: adding/modifying a Reference Segment			
	21	RDG ROI tab: Change the Reference Segment index Base in an embedded RDG			
	22	RDG ROI tab: deleting a Layer			
	23	RDG ROI tab: deleting a Reference Segment			
	24	RDG ROI tab: deleting an ROI			
	25	RDG ROI tab: import genbank sequences into the RDG for automated Ref Segment and feature creation			
	26	RDG NT variants Tab: Edit NT variants in an RDG			
	27	RDG NT variants Tab: Import NT variants from a Tab Delimited Text into RDG			
	28	RDG AA variants Tab: Add amino acid variants to an RDG			
	29	RDG AA variants Tab: Edit AA variants in a RDG			

Table D-2 Access for Admin and Scientist Levels *(continued)*

		Description of access for users of Admin and Scientist levels	Admin	Scientist	Analyst
RDG	30	RDG AA variants Tab: Import AA variants from a tab delimited text file into RDG	Allowed	Allowed	Not Allowed
	31	RDG variant styles tab: configure an RDG in Variant Styles tab			
Library	32	Library: overwriting/appending sequences to an existing library	Allowed	Allowed	Not Allowed
	33	Library: editing sequence data in the library			
	34	Library: exporting data from the library as a Multi-FASTA file			
	35	Library: viewing/editing library types in the Library Type manager			
	36	Library: creating a new sequence library			
Other	37	Sets General Preferences in Options	Allowed	Allowed	Not Allowed
	38	Sets Sequence Collector (Database) Preferences in Options			
	39	Add NT or AA variants from any data view			
	40	Set specimen level analysis settings			
	41	Set project level analysis settings			

Table D-3 Access for Admin, Scientist and Analyst Levels

		Description of access for users of Admin, Scientist and Analyst levels	Admin	Scientist	Analyst
Reports	1	View Reports	Allowed	Allowed	Allowed
	2	View Reports with enabled links back to primary data			
	3	View Reports while editing project			
	4	Export all reports			
	5	Export all customized reports			
	6	Print all reports			
	7	View heterozygous frame shifts links from Mutations Report			
	8	Print a report from the reports manager			
Project View/Display	9	Move sample data from one Specimen to another	Allowed	Allowed	Allowed
	10	Display SQVs and CQVs			
	11	Re-order aligned Specimen consensi			
	12	Change active Layer view			
	13	Show/hide variants that result in silent mutations			
	14	Sort Summary Table in Specimen view			
	15	Display Sample and Consensus Scores			
	16	View Amino Acid tooltips for degenerate codons			
	17	View Amino Acid Alignment in Main Window			
	18	View Library Search Results in Alignment View Identification Pane			

Table D-3 Access for Admin, Scientist and Analyst Levels *(continued)*

		Description of access for users of Admin, Scientist and Analyst levels	Admin	Scientist	Analyst
Project View/Display	19	View electropherogram data as aligned peaks	Allowed	Allowed	Allowed
	20	View all objects in Project Navigator and Main Windows			
	21	View Specimen Layout			
	22	View Specimen-Segment Assembly tab			
	23	View Unassembled data in the Project Navigator and Specimen Views			
	24	View/Navigate through electropherogram snippets			
	25	View/Navigate Specimen Segment electropherogram data			
	26	View a Project/Navigate using the Overview pane			
	27	View Samples in the Sample Manager tab			
	28	View/Navigate alignments using the display toolbar buttons			
Project-Other Controls	29	Apply a new Project Template to an existing Project	Allowed	Allowed	Allowed
	30	Create a new Project from the SeqScape Toolbar			
	31	Delete Samples in Project Navigator			
	32	Delete Specimens in Project Navigator			
	33	Export Project Alignment in FASTA format			
	34	Export Sample data in SEQ, FASTA or AB1 format			
	35	Export Specimen consensus or aligned sample sequences in FASTA format			

Table D-3 Access for Admin, Scientist and Analyst Levels *(continued)*

		Description of access for users of Admin, Scientist and Analyst levels	Admin	Scientist	Analyst
Project-Other Controls	36	Import a Text segment to a Text Specimen	Allowed	Allowed	Allowed
	37	Import Samples to Project			
	38	Import Samples to Project from Database (Sequence Collector)			
	39	Import/create a text-only Specimen			
	40	Open an embedded Settings Object inside a Project			
	41	Open an existing Project			
	42	Print wrapped nucleotide or amino acid Project Alignments			
	43	Save Project from the Menu or Toolbar			
	44	Search for text strings in any sequence data			
Editing	45	Generate an Audit Trail event	Allowed	Allowed	Allowed
	46	Project Alignment view: Change consensus basecalls			
	47	Project Alignment view: Insert or delete a space in a Reference			
	48	Project Alignment view: Insert or delete a space in a Specimen consensus			
	49	Project Alignment view: Insert/delete Consensus bases			
	50	Project Navigator: Rename Specimens			
	51	ROI tab: Rename Segments in RDG			
	52	Specimen view: Change a base in a sample			
	53	Specimen view: Change basecalls in the consensus			

Table D-3 Access for Admin, Scientist and Analyst Levels *(continued)*

		Description of access for users of Admin, Scientist and Analyst levels	Admin	Scientist	Analyst
Editing	54	Specimen view: Change the Clear Range for sample data	Allowed	Allowed	Allowed
	55	Specimen view: Insert or delete a base in a sample			
	56	Specimen view: Insert or delete bases in consensus			
	57	Undo base edits			
SeqScape Manager	58	Open the SeqScape Manager	Allowed	Allowed	Allowed
	59	Save any SeqScape Manager Object			
Library	60	View the Libraries in the SeqScape Manager	Allowed	Allowed	Allowed
	61	View results of Library search in the Project Alignment View			
Analysis Protocol and Settings	62	View the Analysis Protocol	Allowed	Allowed	Allowed
	63	Change basecaller settings in an existing Sample within a Project			
	64	Reconfigure Analysis Defaults inside a Project			
	65	Configure Display Settings in Project or SeqScape Manager			
	66	Analyze data using the BGB without basecalling samples			
	67	Analyze data using the BGB			
	68	Indicate that specific Samples are not to be basecalled			

Table D-3 Access for Admin, Scientist and Analyst Levels *(continued)*

		Description of access for users of Admin, Scientist and Analyst levels	Admin	Scientist	Analyst
Other	69	Browse/Locate data in the file system	Allowed	Allowed	Allowed
	70	Exit SeqScape			
	71	Sort items in columns in any table in SeqScape			
	72	Install SeqScape on a clean system			
	73	Upgrade SeqScape Software v1.0 or v1.1 to v2.0			
	74	Uninstall SeqScape			
	75	Launch SeqScape			
	76	Configure a sample in Data Collection for automated import into SeqScape			

Aligned Variant and FASTA File Format

E

This appendix contains:

About Tab-Delimited Files

You can import variants into the ABI PRISM® SeqScape® Software v2.0 if they are in the format of a tab-delimited text file.

Creating a Variant Text File

SeqScape software tab-delimited text files must conform to the following rules:

- One variant per line
- Six tab-delimited column headings:
  - Type
  - NT position
  - Reference
  - Variant
  - Style
  - Description

An example is provided in Figure E-1 on page E-3:

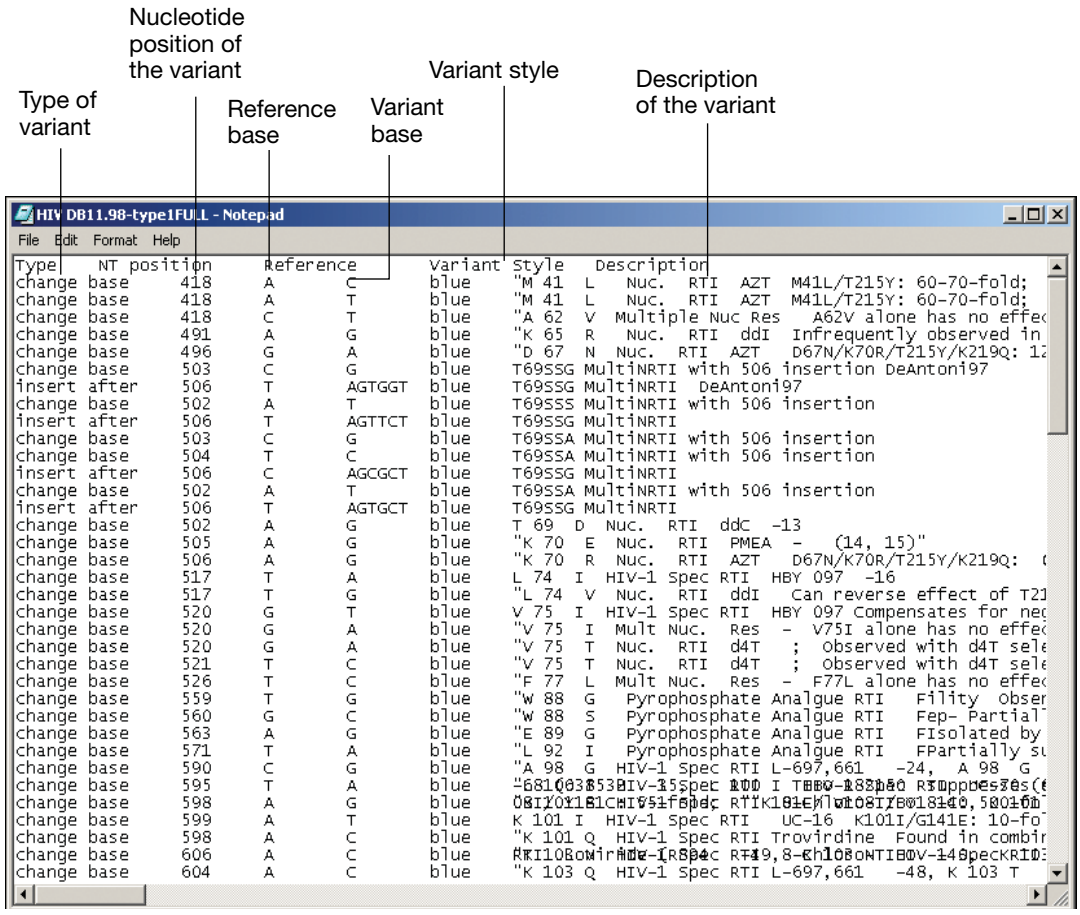


Figure E-1 Sample of Variant Tab-Delimited Text Format

FASTA File Format

Note: The information on FASTA was obtained from http://www.ncbi.nlm.nih.gov/BLAST/fasta.html

FASTA Format Description

A sequence in FASTA format begins with a single-line description, followed by lines of sequence data. The description line is distinguished from the sequence data by a greater-than (>) symbol in the first column.

FASTA Format Example

An example sequence in FASTA format is as follows:

>HIV HXB2 Prt-RT1(1-320)
cctcaggtcactctttggcaacgacccctcgtcacaataaagataggggggcaactaaaggaag
ctctattagatacaggagcagatgatacagtattagaagaaatgagtttgccaggaagatggaaa
ccaaaaatgatagggggaattggaggttttatcaaagtaagacagtatgatcagatactcatagaa
atctgtggacataaagctataggtacagtattagtaggacctacacctgtcaacataattggaaga
aatctgttgactcagattggttgcactttaaattttcccattagccctattgagactgtaccagtaaaat
taaagccaggaatggatggcccaaaagttaaacaatggccattgacagaagaaaaaataaaag
cattagtagaaatttgtacagagatggaaaaggaagggaaaatttcaaaaattgggcctgaaaat
ccatacaatactccagtatttgccataaagaaaaaagacagtactaaatggagaaaattagtagat
ttcagagaacttaataagagaactcaagacttctgggaagttcaattaggaataccacatcccgca
gggttaaaaaagaaaaaatcagtaacagtactggatgtgggtgatgcatatttttcagttcccttag
atgaagacttcaggaagtatactgcatttaccatacctagtataaacaatgagacaccagggatta
gatatcagtacaatgtgcttccacagggatggaaaggatcaccagcaatattccaaagtagcatg
acaaaaatcttagagccttttagaaaacaaaatccagacatagttatctatcaatacatggatgattt
gtatgtaggatctgacttagaaatagggcagcatagaacaaaaatagaggagctgagacaacat
ctgttgaggtggggacttaccacaccagacaaaaaacatcagaaagaacctccattcctttggat
gggttatgaactccatcctgataaatggacagtacagcctatagtgctgccagaaaaagacagct
ggactgtcaatgacatacagaagttagtggggaaattgaattgggcaagtcagatttacccaggg
attaaagtaaggcaattatgtaaactccttagaggaaccaaagcactaacagaagtaataccacta
acagaagaagcagagctagaactggcagaaaacagagagattctaaaagaaccagtacatgg
agtgtattatga

FASTA Codes

Sequences are expected to be represented in the standard IUB/IUPAC amino acid and nucleic acid codes, with the following exceptions:

- Lower-case letters are accepted and are mapped into uppercase
- In amino acid sequences, U and * (asterisk) are acceptable letters (see below)

Note: Although FASTA codes allow a hyphen or dash to represent a gap in nucleotide sequences, this practice is not acceptable for using FASTA format in SeqScape software.

Before importing a sequence, any numerical digits or spaces in the sequence need to be either removed or replaced by appropriate letter codes (for example, N for unknown nucleic acid residue or X for unknown amino acid residue).

Supported Nucleic Acid Codes

Table E-1 Accepted Nucleic Acid Codes:

Character …	Codes for …
A	Adenosine
C	Cytidine
G	Guanine
T	Thymidine
U	Uridine
R	GA (purine)
Y	TC (pyrimidine)
K	GT (keto)
M	AC (amino)
S	GC (strong)
W	AT (weak)
B	GTC
D	GAT
H	ACT
V	GCA
N	AGCT

Supported Amino Acid Codes

Table E-2 Accepted Amino Acid Codes:

Character …	Codes for …
A	Alanine
B	Aspartate or asparagine
C	Cystine
D	Aspartate
E	Glutamate
F	Phenylalanine
G	Glycine
H	Histidine
I	Isoleucine
K	Lysine
L	Leucine
M	Methionine
N	Asparagine
P	Proline
Q	Glutamine
R	Arginine
S	Serine
T	Threonine
U	Selenocysteine
V	Valine
W	Tryptophan
Y	Tyrosine
Z	Glutamate or glutamine

Table E-2 Accepted Amino Acid Codes: *(continued)*

Character ...	Codes for ...
X	Any
*	Translation stop
-	Gap of indeterminate length

Software Warranty Information

F

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 ABI PRISM® SeqScape® Software Version 2.0 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.

The foregoing provisions set forth Applied Biosystems' sole and exclusive representations, warranties, and obligations with respect to its products, and Applied Biosystems makes no other warranty of any kind whatsoever, expressed or implied, including without limitation, warranties of merchantability and fitness for a particular purpose, whether arising from a statute or otherwise in law or from a course of dealing or usage of trade, all of which are expressly disclaimed.

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The remedies provided herein are the buyer's sole and exclusive remedies. Without limiting the generality of the foregoing, in no event shall Applied Biosystems be liable, whether in contract, tort, warranty**,** or under any statute (including without limitation any trade practice, unfair competition**,** or other statute of similar import) or on any other basis, for direct, indirect, punitive, incidental, multiple, consequential, or special damages sustained by the buyer or any other person or entity, whether or not foreseeable and whether or not Applied Biosystems is advised of the possibility of such damages, including without limitation, damages arising from or related to loss of use, loss of data, failure or interruption in the operation of any equipment or software, delay in repair or replacement, or for loss of revenue or profits, loss of good will, loss of business, or other financial loss or personal injury or property damage.

No agent, employee, or representative of Applied Biosystems has any authority to modify the terms of this Limited Warranty Statement or to bind Applied Biosystems to any affirmation, representation, or warranty concerning the product that is not contained in this Limited Warranty Statement, and any such modification, affirmation, representation, or warranty made by any agent, employee, or representative of Applied Biosystems will not be binding on Applied Biosystems unless in a writing signed by an executive officer of Applied Biosystems.

This warranty is limited to the buyer of the product from Applied Biosystems and is not transferable.

Some countries or jurisdictions limit the scope of or preclude limitations or exclusion of warranties, of liability, such as liability for gross negligence or wilful misconduct, or of remedies or damages, as or to the extent set forth above. In such countries and jurisdictions, the limitation or exclusion of warranties, liability, remedies or damages set forth above shall apply to the fullest extent permitted by law, and shall not apply to the extent prohibited by law.

Glossary

ABI basecaller	An algorithm used in earlier versions of ABI PRISM® DNA Sequencing Analysis and ABI PRISM® SeqScape® Software.
administration	The functions of SeqScape software relating to installing, removing, or updating the application.
aligned allele library	A collection of aligned sequences that are all variations of the same sequence. This is the only type of library supported in SeqScape v2.0. An aligned allele library differs from a library of diverse sequences such as a library of different gene sequences, and is also different from a library of unaligned sequences.
alignment	The aligned reference sequence together with the aligned specimen consensus sequences.
alignment display	A table of IUB codes, space characters, blanks, and dots showing how the sequences within a project are aligned.
alignment score	The number of mismatches between the aligned reference and the aligned consensus sequence for a given specimen.
allele	An alternative form of a genetic locus.
analysis	The complete procedure that SeqScape Software performs in a batch-wise manner on sample data.
analysis defaults	The default analysis settings that are stored in a project template.
analysis protocol	The default settings (basecalling, mixed base identification, clear range and trimming, and filtering) that govern sample analysis.
analysis settings	The parameters that govern the basecalling, trimming, filtering, and assembly of the analysis.

assembly	The set of aligned and overlapping sample data that result from the sequencing of one PCR product or clone.
Assembly view	Shows the specimen consensus sequence as well as the aligned sample sequences. Electropherograms and quality values can also be viewed.
basecaller	An algorithm that determines the bases within a sequence during analysis. There are two types of basecallers: KB basecallers and ABI basecallers.
clear range	The region of sequence that remains after excluding the low-quality or error-prone sequence at both the 5' and 3' ends.
comparison	The relationship between the aligned specimen consensi and the reference sequence and the associated reference data.
consensus quality values	See quality values.
consensus caller	The analysis algorithm that is responsible for generating an accurate consensus sequence with per-base quality values.
consensus sequence	The output of the assembly from a biologically related group of samples.
constant position	A position in the library alignment that is identical for every allele in the library. See polymorphic position.
constant position error	A position in a specimen consensus sequence that corresponds to a constant position in the library and that disagrees with the library at that position.
contig	The set of aligned and overlapping sample data that results from the sequencing of one PCR product or clone. Also known as an assembly.
crucial position	A position in a specimen consensus sequence that differs among the set of matches returned after a library search.
display settings	The parameters that govern the display of the data and results.

DyeSet/Primer file	The DyeSet/Primer file compensates for the mobility differences between the dyes and primers and corrects the color-code changes due to the chemistry used to label the DNA. DyeSet/Primer files are sometimes referred to as mobility files.
export	Moving the data or settings from inside the SeqScape Software Data Store to outside the SeqScape Software Data Store either in .ctf or .txt format.
FASTA format	A standard text-based file format for storing one or more sequences.
filtered sample sequence	A sample that has been processed by the basecaller/factura/filter algorithms of the pipeline.
genotype library	A library where the allele sequences are either pure-base or mixed-base sequences. When searching against a genotype library, SeqScape attempts to find the best matches to the consensus sequence without trying different allele combinations. **Note:** This term is not used by SeqScape software.
haplotype library	A library where the allele sequences are completely pure-base sequences. When searching against a haplotype library, SeqScape attempts to combine haplotypes two at a time to find the best genotype match to the consensus sequence.
IUB/IUPAC	International Union of Biochemistry/International Union of Pure and Applied Biochemistry. More information can be found at http://www.chem.qmw.ac.uk/iubmb/misc/naseq.html#300.
KB basecaller	A new algorithm that calculates mixed or pure bases and determines sample quality values.
layout view	Shows the layout of the sample assembly with arrows indicating the placement and orientation of samples.
library match	The name of one allele or the combination of two alleles (depending on the library type) that agree closely with the specimen consensus sequence.

nibbler	The algorithm that sets the clear range for each sample using the clear range settings specified in the analysis settings.
polymorphic position	A position in the library alignment that differs for some alleles in the library. See constant position.
project	A group of related sequences that share the same reference or for which there is no explicit reference.
project summary sequence	A summary of the alignment of the specimen consensi.
project template	Contains an RDG, analysis defaults, display settings, and output settings.
quality values	Measure of certainty of the basecalling and consensus-calling algorithms. Higher values correspond to lower chance of algorithm error. Sample quality values refer to the per-base quality values for a sample, and consensus quality values are per-consensus quality values.
reference	A nucleotide string that has the following attributes: it is contiguous, it is not editable, its orientation determines the project orientation, and it is stored in the RDG.
reference associated data	The things that are related or assigned to a particular base or ranges of bases on a reference. There are two types of reference associated data: structural and variant.
Reference Data Group (RDG)	The data that contains the reference and the reference associated data.
Report Manager	A window that contains nine separate reports detailing the success or failure of various portions of the analysis, statistics, mutations, AA variants, and library search information.
sample data	The output of a single lane or capillary on a sequencing instrument that will be input into SeqScape Software.

Sample Manager	A window that displays sample file name, name and specimen; last used basecaller and DyeSet/Primer files; calculated basecalling results (spacing, peak 1, start and stop); and assembly status. The sample name, basecaller, and/or DyeSet/Primer file can be changed here.
sample quality values	See quality values.
sample score	The average of the per-base quality values for the bases in the clear range sequence for the sample.
sample view	A view in the SeqScape software where you can see attributes of each AB1 file including its annotation, sequence, features, raw data, and electropherogram data.
segment	A contiguous segment of the reference sequence corresponding to a single contiguous DNA sequence.
SeqScape Manager	The software component that manages the following settings: SeqScape Software projects, project template, RDG, analysis defaults, and display settings.
space character	A character in an aligned sequence is either an IUB code or space, perhaps shown as a dash (-). A space indicates a deleted base in this string or, equivalently, an inserted base in one of the other aligned strings.
specimen	The container that holds all the sample data as assembled contigs from a biological source or PCR product.
specimen (consensus) quality value	See quality values.
specimen (consensus) score	The average overall of the consensus quality values in the consensus sequence.
specimen consensus sequence	The output of the consensus-calling algorithm from a biologically related group of samples.
specimen report	A concatenated list of all the reported information on a per specimen basis.

specimen view	A view in SeqScape software where you can see the consensus sequence and all sample files that were used to create that consensus sequence.
summary sequence	The summary consensus sequence for the entire library alignment. Pure positions in the summary sequence correspond to *constant positions* and mixed-base positions in the summary sequence correspond to *polymorphic positions*.

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