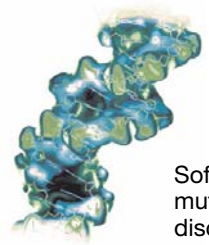
Applied Biosystems SeqScape® Software

The Mutation Detection Software Solution

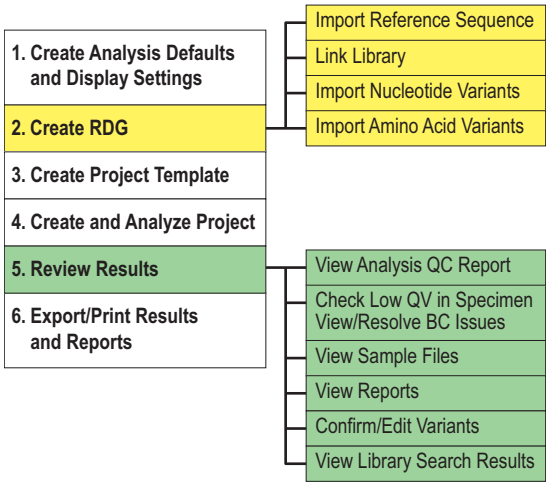


SeqScape Software Version 2.5

Applied Biosystems SeqScape Software v2.5 is expressly designed for mutation detection and analysis, SNP discovery and validation, pathogen subtyping, allele identification, and sequence confirmation.

Workflow for Analyzing and Reviewing Data

There are six main steps to analyze and review your data:



All analysis in SeqScape software occurs in a project.

Perform steps 1 to 3 only when you need to create a new project template.

Perform steps 4 to 6 each time new data is being analyzed.

For more information on a step, refer to the *SeqScape® Software User Guide* (PN 4359442).

Input Files

SeqScape software uses the following file types:

File Extension and Format	Description
Files Used for Analysis	
.ab1 (ABI format)	A file generated from Applied Biosystems 3130/3130*xl* and 3730/3730*xl* Analyzers and ABI PRISM® 310, 3700, 3100, and 3100-*Avant* Analyzers and 377 Sequencers.
.txt or .fsta (FASTA format)	A file containing a single sequence in FASTA format.
Files Used for the Reference Data Group (RDG)	
.ab1 (ABI format)	A file generated from Applied Biosystems 3130/3130*xl* and 3730/3730*xl* Analyzers and ABI PRISM® 310, 3700, 3100, and 3100-*Avant* Analyzers and 377 Sequencers.
.txt or .fsta (FASTA format)	A file containing a single sequence in FASTA format.
.gb (Genbank format)	A text file downloaded from the NCBI database, then saved with the .gb extension.
Files Used for the Nucleotide Variants in the RDG	
.fsta (FASTA format)	A text file containing a set of aligned sequences in FASTA format.
.txt (Tab-delimited)	A tab-delimited text file that has one variant per line and eight column headings: Type, ROI, NT position, Reference, Variant, Style, Description, and Used by all ROIs.
Files Used for the Amino Acid Variants in the RDG	
.txt (Tab-delimited)	A tab-delimited text file that has one variant per line and seven column headings: Type, Layer, AA position, Reference, Variant, Style, and Description

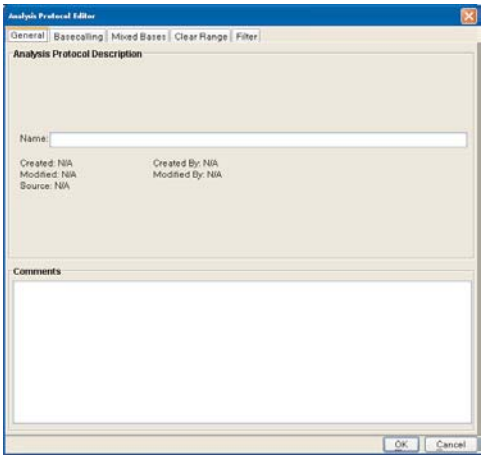
QUICK REFERENCE CARD

Step 1: Create Analysis Defaults and Display Settings.

1A. Select **Tools** > **SeqScape Manager**.

1B. Create default analysis settings:

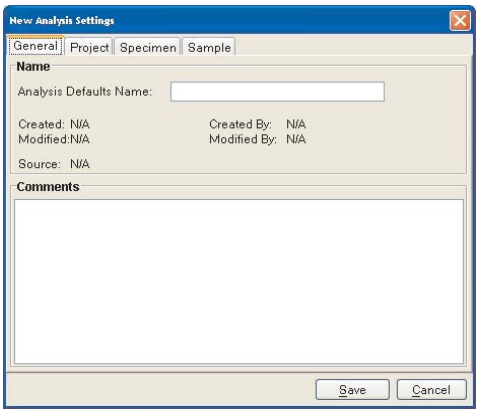
a. Select the **Analysis Protocols** tab, then click **New**.



b. Complete the tabs to specify a name and to select a basecaller, mixed base settings, clear range, and filter settings.

c. Click **OK**.

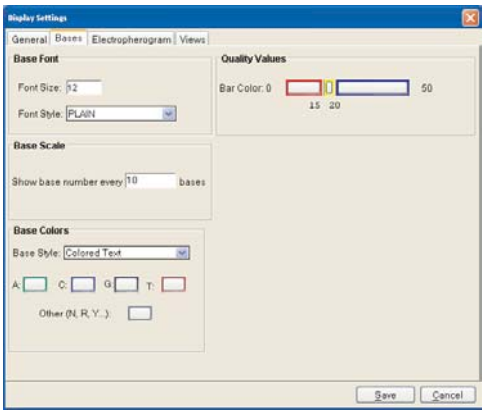
d. At the SeqScape Manager main window, select the **Analysis Defaults** tab, then click **New**.



e. Complete the tabs to specify your settings, then click **Save**.

1C. Create default display settings:

a. Select the **Display Settings** tab, then click **New**.



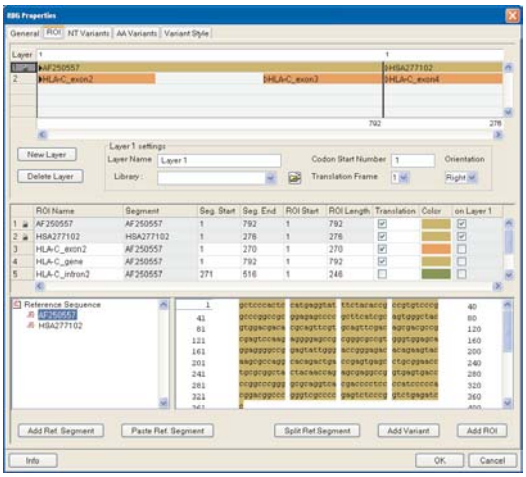
b. Complete the tabs to specify a name and display characteristics for quality values, electropherograms, and views.

c. Click **Save**.

Step 2: Create an RDG.

2A. In the SeqScape Manager, select the **Reference Data Group** tab, then click **New**.

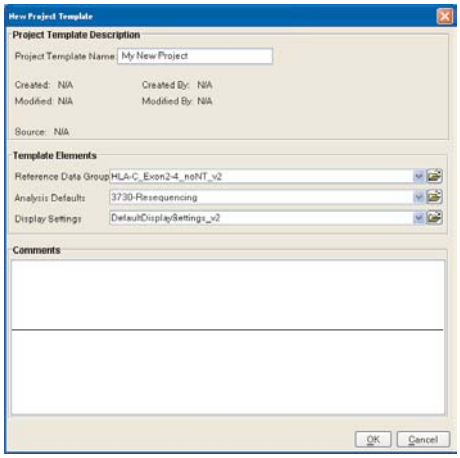
2B. Step through the tabs to name the RDG, import the reference sequences, define layers and ROIs, link allele libraries (optional), designate the start codon, import NT and AA variants, and choose the variant display styles.



2C. Click **OK**.

Step 3: Create a Project Template.

3A. In the SeqScape Manager, Select the **Project Templates** tab, then select **New**.



3B. Enter a name for the new template, then in the drop-down lists select the RDG, analysis defaults and display settings files you created in the previous steps.

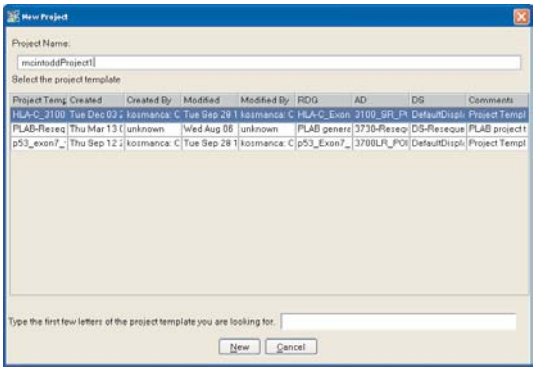
3C. Click **OK**.

3D. Close the SeqScape Manager.

Step 4: Create and Analyze a Project.

4A. Create a project:

a. Click (New Project).



b. Enter a new name in the Project Name field, select the project template you created in step 3, then click **New**.

4B. Add samples to the project automatically:

This requires that you have a common text delimiter in each sample file name or sample name. All the samples to be imported automatically must be stored in a single folder.

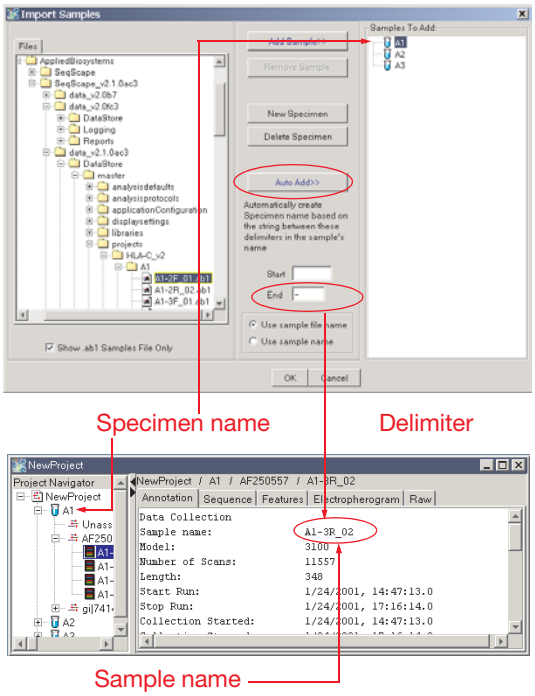
a. Click (Import Samples To Project).

b. Enter a delimiter in the Specimen name delimiter field.

c. If you are using the sample name, deselect the **Use sample file name** check box.

d. Select the folder containing the sample files to add.

e. Click **Auto Add**. The specimens are created and the samples are imported automatically.



f. When you finish adding samples, click **OK**.

4C. Click (Analyze Samples).

Step 5: Review the Results.

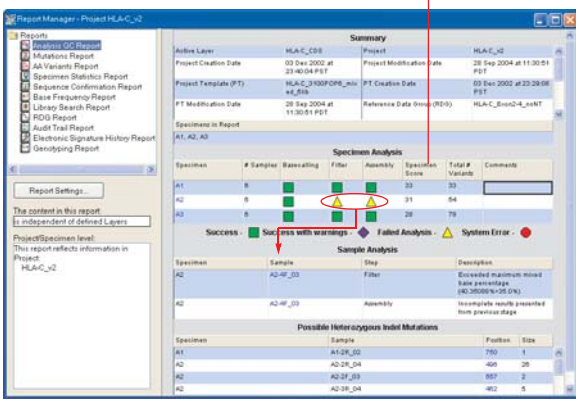
5A. Open the project of interest, then select the project name in the Navigation pane.

5B. Select a layer in the Active Layer drop-down list.

5C. Check for analysis failures:

a. Click (Report Manager), then select **Analysis QC Report**.

Ctrl-drag to reorder columns



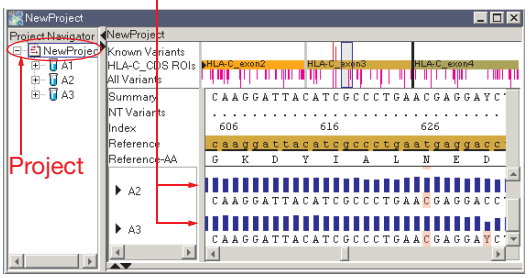
b. If yellow triangles or red circles are present, verify the data quality and analysis settings.

c. Correct the analysis settings, then reanalyze the data, if necessary.

5D. Review the data in the project:

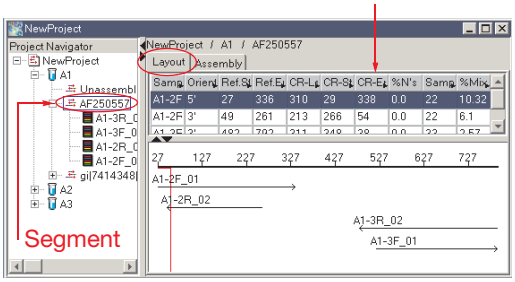
a. Select a layer, then check bases with low quality values. Verify the basecalls of the consensus sequence.

Quality values



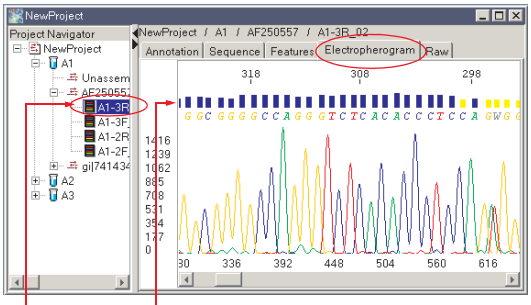
b. Select a segment, then select the **Layout** tab to view the segment sequence layout.

Double-click header to sort in ascending or descending order



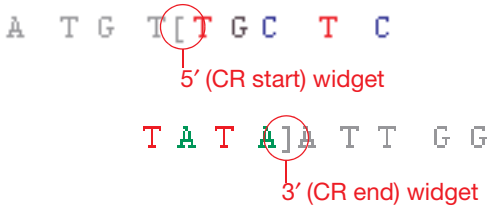
c. Select a segment, then select the **Assembly** tab to view the segment sequence assembly.

d. Select a sample, then select the desired tab to view the sample results. Shown below is the electropherogram data.



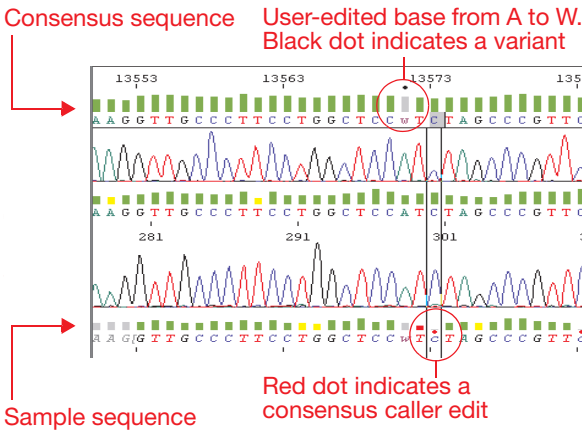
5E. Adjust the clear range by:

- Fewer than 50 bases – Select a clear range widget, **[** or **]**, then drag the widget between two bases that represents the new location.



- More than 50 bases – Place the pointer between two bases that represents the new location, right-click, then select the item in the shortcut menu to set the start or end of the clear range.

5F. Edit results by changing, deleting, and adding bases or adding and deleting spaces in a specimen consensus or sample sequence. The changes shown below were done either automatically by the consensus-calling algorithm or manually by the user.



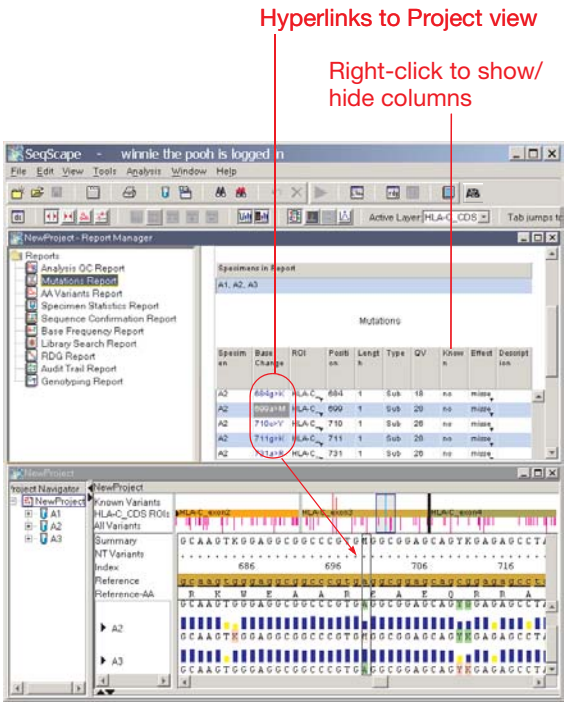
5G. Review the Mutations and AA Variants reports to confirm or edit variants:

a. Select the project name in the navigation pane, then select a layer in the Active Layer drop-down list.

b. Click (Report Manager), then select the **Mutations Report** or **AA Variant Report**.

c. Select **Window** > **Tile**.

d. Click a hyperlink (blue text) in the report, then view the data in the project view.



5H. Review other reports of interest.

5I. Use the Library Search report to view and edit constant position errors, if applicable:

a. Select the project name in the Navigation pane, then select an active layer.

b. Click (Report Manager), then select the **Library Search Report**.

c. Select **Window** > **Tile**.

d. In the Constant Position Errors table of the report, select a position (hyperlinked blue text), then view and edit the data in the project view.

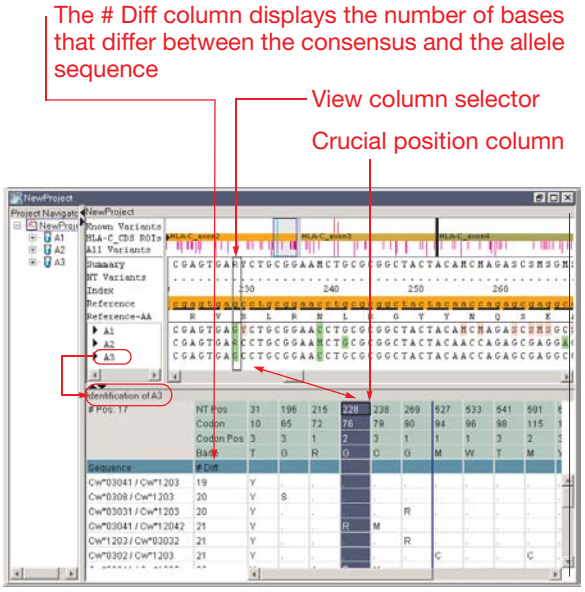
5J. Use the Identification pane to view and edit crucial positions, if applicable:

a. Select the project name in the Navigation pane, then select a layer in the Active Layer drop-down list.

b. Select a base in the consensus sequence to populate the Identification pane.

c. Use the split bar to adjust the height of the Identification pane.

d. Click  (View Column Selector).



e. Select a crucial position in the Identification pane. The corresponding consensus base is highlighted in the view column selector in the Project view.

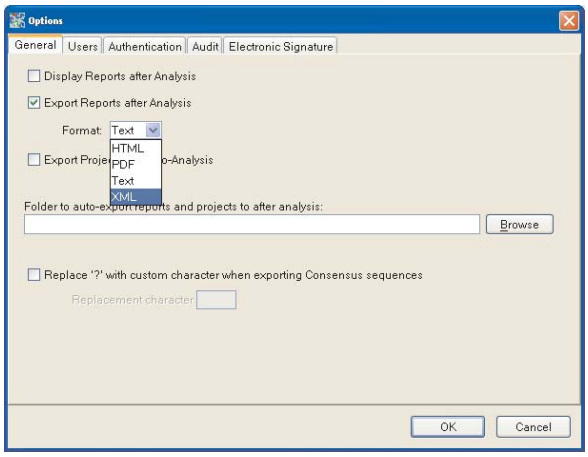
f. View and edit the data in the Project view.

Note: The crucial positions in the Identification pane and the Project view are hyperlinked to each other. Therefore, sequence edits are automatically updated in the library search results in the Identification pane.

Step 6: Export/Print the Results and Reports.

Reports can be automatically exported after analysis.

6A. Select **Tool** > **Options**.



6B. Complete the General tab:

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 in the drop-down list.

c. Define a default location to save the files.

d. Click **OK**.

Note: To manually export and/or print files, refer to Chapter 9 of the *SeqScape Software User Guide*.