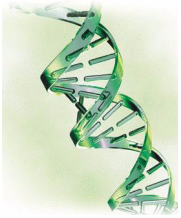


Applied Biosystems SeqScape® Software 3

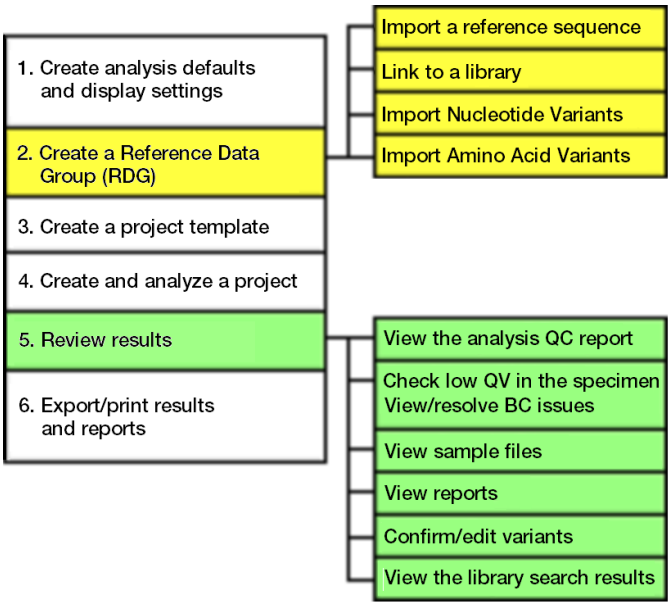
Quick Reference Card

Publication Part Number 4474241 **Revision Date** 15 January 2012 Rev. A

Applied Biosystems SeqScape® Software 3 is 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 Applied Biosystems 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 are analyzed.

For more information, refer to the *Applied Biosystems SeqScape® Software 3 User Guide* (Part no. 4359442).

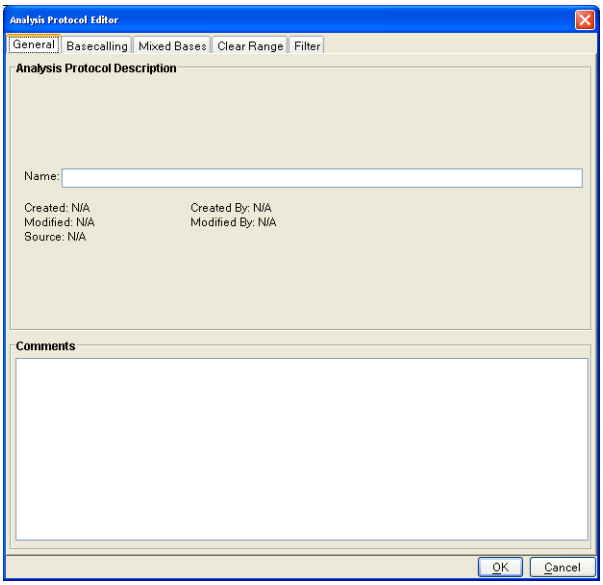
Input files

Applied Biosystems SeqScape® Software uses the following file types:

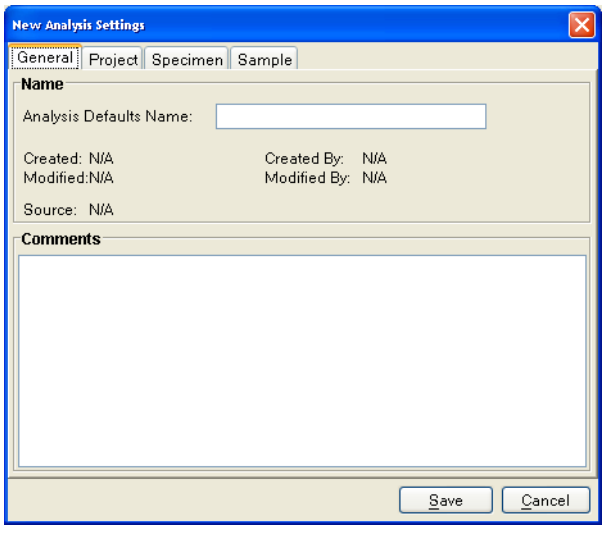
File extension and format	Description
Files that are used for Analysis	
.ab1 (ABI format)	A file that is generated from any of the following Life Technologies instruments: • 3130/3130*xl* Genetic Analyzers • 3730/3730*xl* Genetic Analyzers • 310 Genetic Analyzer • 3100/3100-*Avant* Genetic Analyzers • 3500 Dx/3500xL Dx Genetic Analyzers • 3500/3500xL Genetic Analyzers
.txt or .fsta (FASTA format)	A file containing a single sequence in FASTA format.
Files that are used for the RDG	
.ab1 (ABI format)	A file that is generated from any of the following Life Technologies instruments: • 3130/3130*xl* Genetic Analyzers • 3730/3730*xl* Genetic Analyzers • 310 Genetic Analyzer • 3100/3100-*Avant* Genetic Analyzers • 3500 Dx/3500xL Dx Genetic Analyzers • 3500/3500xL Genetic Analyzers
.txt or .fsta (FASTA format)	A file containing a single sequence in FASTA format.
.gb (Genbank format)	A text file that is dowloaded from the NCBI database, then saved with the .gb extension.
Files that are 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 that are 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, ROI, NT position, Reference, Variant, Style, and Description.

Step 1: Create analysis defaults and display settings

1. Select **Tools ▸ SeqScape Manager**.
2. Create default analysis settings:
   a. Select the **Analysis Protocols** tab, then click **New**.
   b. Complete the tabs by specifying a name and selecting a basecaller, mixed base settings, clear range, and filter settings.
   c. Click **OK**.

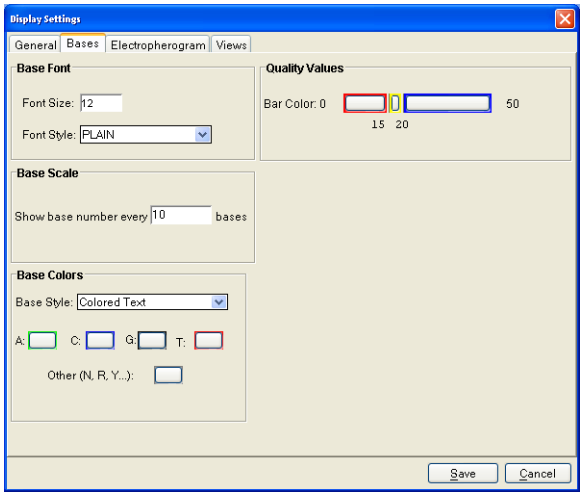


   d. In the SeqScape Manager main window, select the **Analysis Defaults** tab, then click **New**.



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

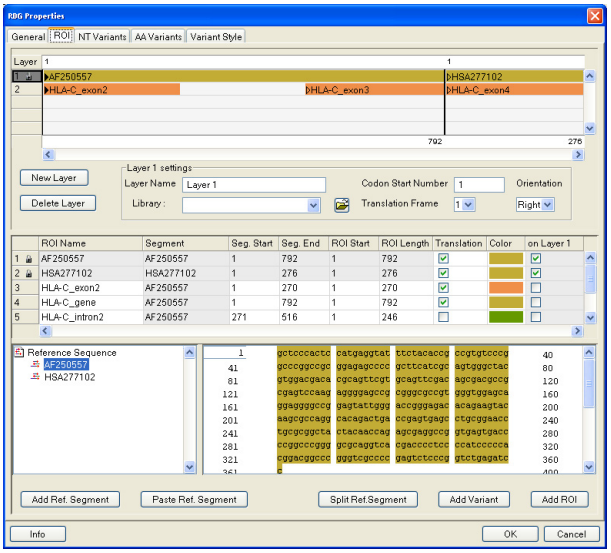
3. Create default display settings:
   a. Select the **Display Settings** tab, then click **New**.



   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

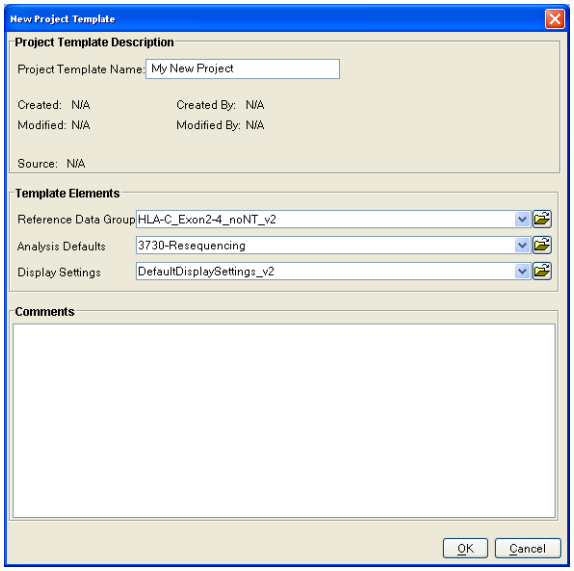
1. In SeqScape Manager, select the **Reference Data Group** tab, then click **New**.
2. Select the tabs in succession to name the RDG, import the reference sequences, define layers and ROIs, link allele libraries (optional), designate the start codon, import NT and AA variants, and select the variant display styles.



3. Click **OK**.

Step 3: Create a project template

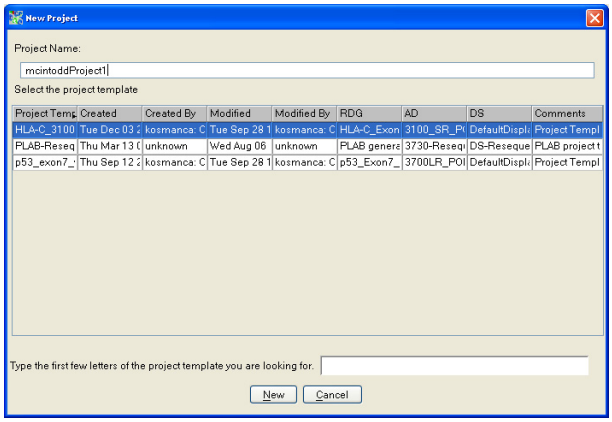
1. In SeqScape Manager, select the **Project Templates** tab, then select **New**.



2. Enter a name for the new template, then, in the drop-down lists, select the same RDG, analysis defaults, and display settings files that you created in the previous steps.
3. Click **OK**.
4. Close SeqScape Manager.

Step 4: Create and analyze a project

1. Create a project:
   a. Click (New Project).

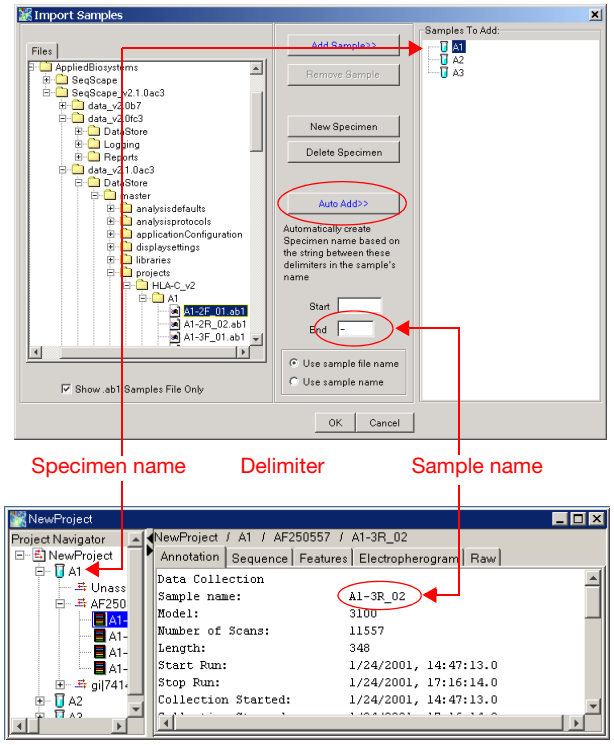


   b. Enter a new name in the Project Name field, select the project template that you created in "Step 3:Create a project template", then click **New**.

2. Add samples to the project automatically:

   (This requires that you have a common text delimiter in each sample file name or sample name, and that all the samples to be imported automatically are stored in a single folder.)

   a. Click (Import Samples To Project).
   b. Enter a delimiter in the Specimen name delimiter field.
   c. If you use 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.

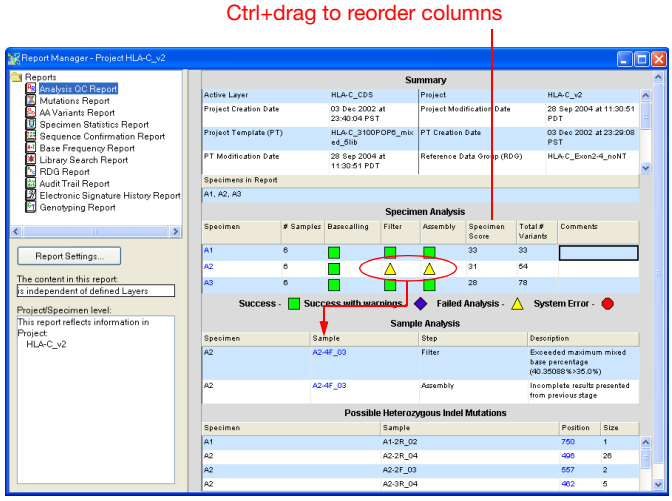


   f. When you finish adding samples, click **OK**.

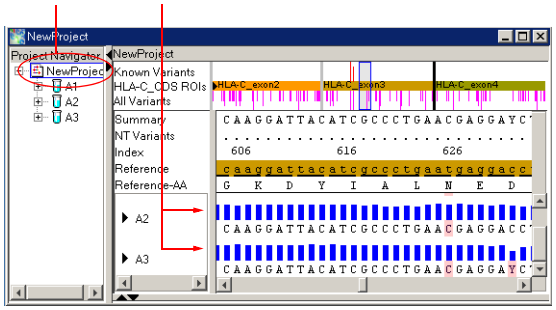
3. Click (Analyze Samples).

Step 5: Review the results

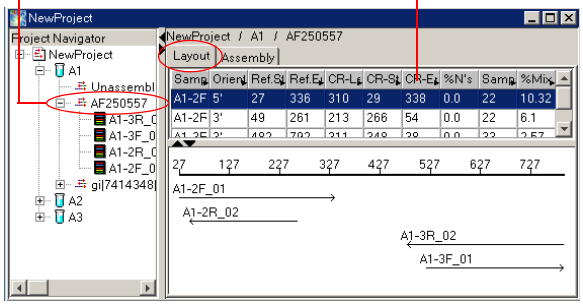
1. Open the project of interest, then select the project name in the navigation pane.
2. Select a layer in the **Active Layer** drop-down list.
3. Check for analysis failures:
   a. Click (Report Manager), then select **Analysis QC Report**.



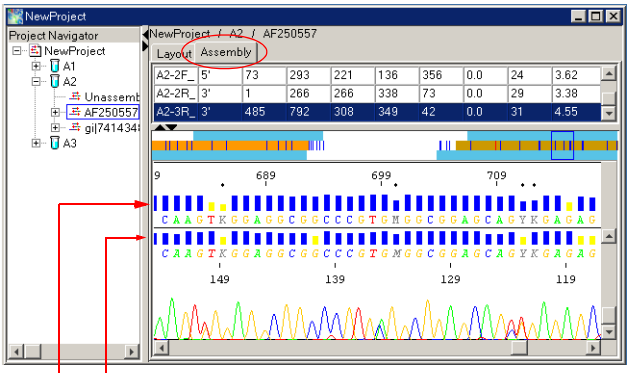
   b. If blue diamonds, yellow triangles, or red circles appear, check the data quality and analysis settings.
   c. Correct the analysis settings, then reanalyze the data if necessary.
4. Review the data in the project:
   a. Select a layer, then observe bases with low quality values. Verify the basecalls of the consensus sequence.



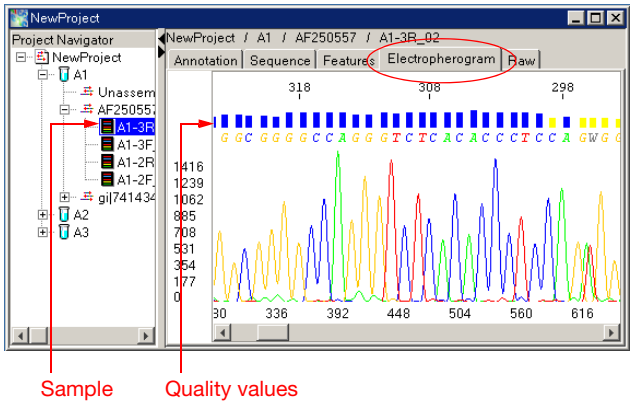
   b. Select a segment, then select the **Layout** tab to view the segment sequence layout.



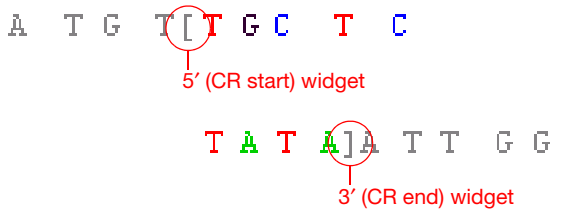
   c. Select a segment, then select the **Assembly** tab to view the segment sequence assembly.



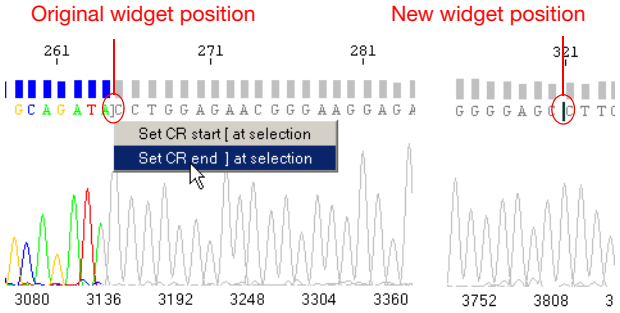
   d. Select a sample, then select the desired tab to view the sample results. Electropherogram data is shown below.



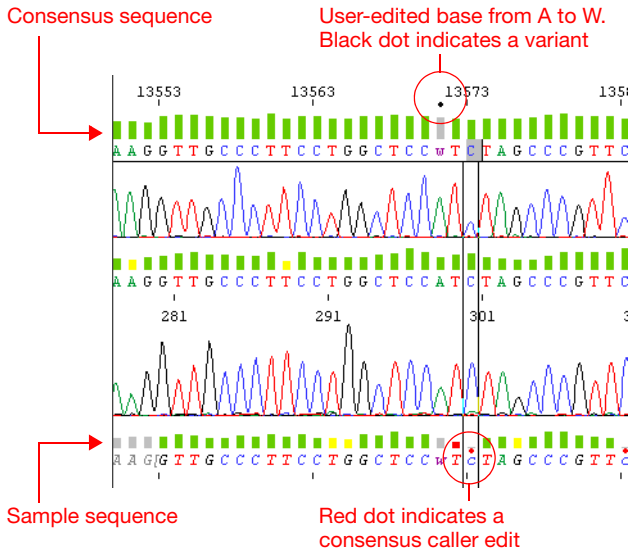
5. Adjust the clear range by the number of bases:
   - Fewer than 50 bases: Select the clear range widget, then click and drag the widget between two bases that represent the new location. The 5' widget '[' and the 3' widget ']' are shown below, circled in red.



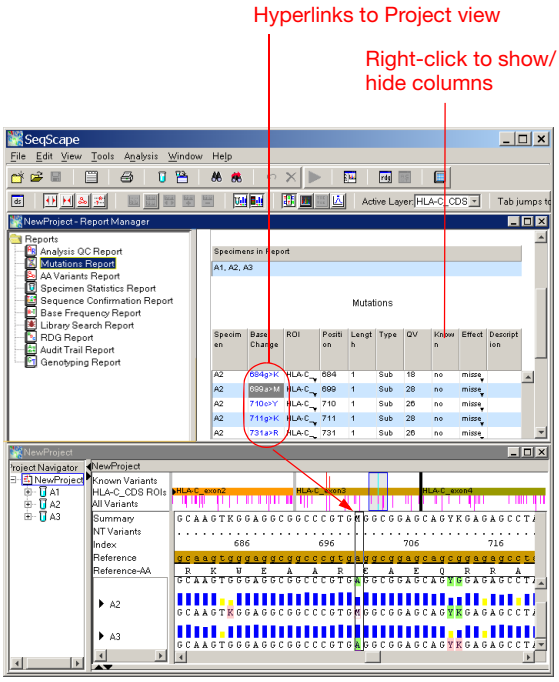
   - More than 50 bases: Place the pointer between two bases that represent the new location, right-click, then select the item in the shortcut menu to set the start or end of the clear range.



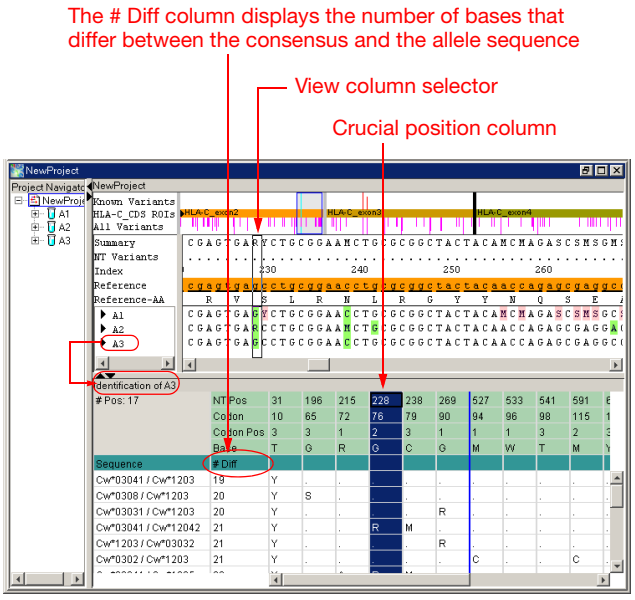
6. Edit results by changing, deleting, and adding bases or adding and deleting spaces in a specimen consensus or sample sequence. The changes shown below were done either automatically by the consensus-calling algorithm, or manually by the user.



7. Review the Mutations and AA Variants reports to verify 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 either **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.



8. Review other reports of interest.
9. 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.
10. 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 selected in the view column selector in the Project view.

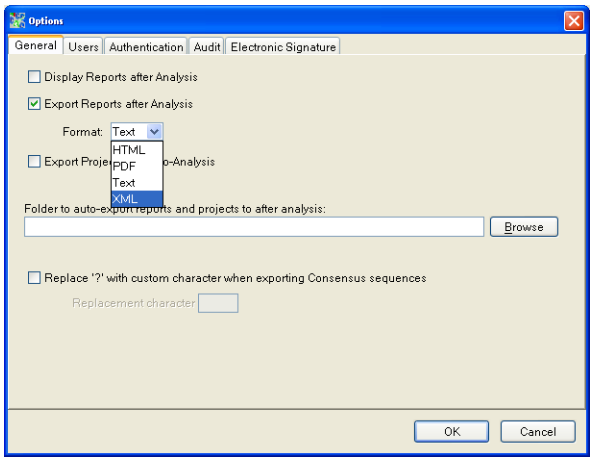
f. View and edit the data in the Project view.

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

After analysis, you can export reports automatically.

1. Select **Tools ▸ Options**.



2. In 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. Specify a default location to save files.

   d. Click **OK**.

Note: To manually export and/or print files, refer to the instructions in the *SeqScape Software 3 User Guide*.

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