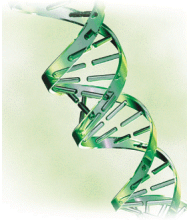


SeqScape® Software Version 2.7 Workflow

Quick Reference Guide

You can use Applied Biosystems SeqScape® Software v2.7 to detect and analyze mutations, discover and validate SNPs, subtype pathogens, identify alleles, and confirm sequences.

This Quick Reference Guide describes how to use the software to perform these functions.

Creating a Project (page 2)

Reviewing Data and Results (page 9)

Changing Analysis Settings (page 15)

Exporting and Printing (page 20)

Creating a Project

All analyses in SeqScape® software occur in a project. Before you can create a project you must create a project template that contains:

- A reference data group (RDG)
- Analysis default settings
- Display settings

After you create a project template, you can use it for future projects.

IMPORTANT! VariantSEQr™ customers must go directly to Add sample files to the project template on page 7.

Creating a project requires you to:

1. Import a reference sequence

2. Define analysis and display settings

3. Create a project template

4. Add sample files to the project template

5. Analyze the data

Step 1: Import a reference sequence

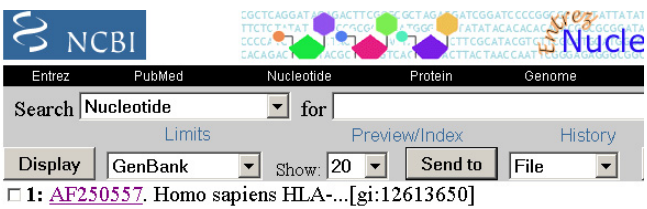
IMPORTANT! A reference sequence can be any of the following formats:

- .txt, ab1, .seq, .fsta files
- Genbank format files
- Aligned sequences in .fsta (FASTA format)

This procedure uses a Genbank file for the reference data. Before you begin, download a Genbank file, then create a known variants table.

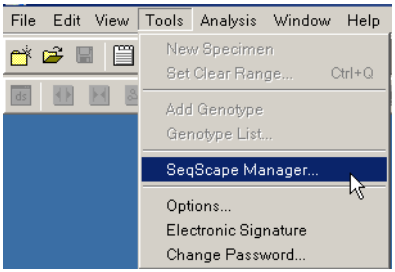
Note: If you download a Genbank file in the NCBI web interface: In the Display drop-down list, select **Genbank**, then In the **Send to** list, select **File.**

This action saves the file as a text file that can be imported.

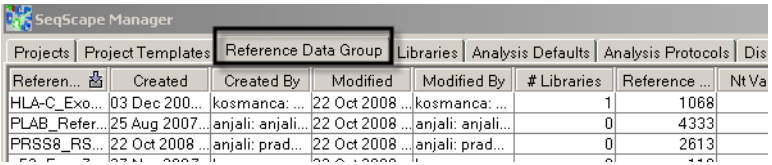


To import a Genbank file as your reference sequence:

1. In the SeqScape® software, select **Tools ▸ SeqScape Manager**.



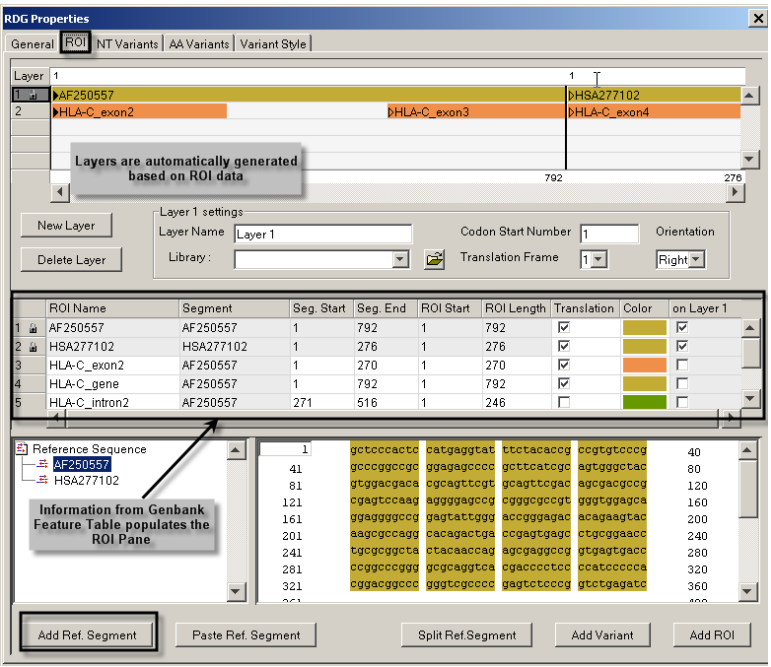
2. Select the **Reference Data Group** tab.
3. Click **New**, then enter a name.



4. Select the **ROI** (Regions of Interest) tab.

   Note: For information on any of the four panes in the ROI tab (Layer, ROI, Reference Sequence, Reference Segment), click **Info**.

5. Click **Add Ref. Segment**.



6. Select the Genbank file, then click **Import**.

   When the Genbank file is imported, information from the feature table populates the ROI table in the SeqScape® software and layers are automatically created.

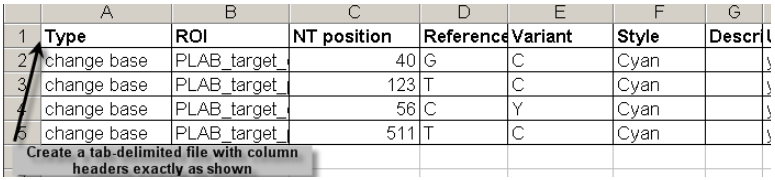
```
FEATURES             Location/Qualifiers
     source          1..792
                     /organism="Homo sapiens"
                     /db_xref="taxon:9606"
                     /map="6p21.3"
     gene            <1..>792
                     /gene="HLA-C"
                     /allele="HLA-Cw*0802"
     mRNA            join(<1..270,517..>792)
                     /gene="HLA-C"
                     /product="HLA-C class I antigen"
     CDS             join(<1..270,517..>792)
                     /gene="HLA-C"
                     /codon_start=3
                     /product="HLA-C class I antigen"
                     /protein_id="AAG53742.1"
                     /db_xref="GI:12483747"
                     /translation="SHSMRYFYTAVSRPGRGEPRFIAVGYVDDTQFVQFDSDAASI
                     EPRAPWVEQEGPEYWDRETQKYKRQAQTDRVSLRNLRGYYNQSEAGSHTLQRMYGC
                     GPDGRLLRGYNQFAYDGKDYIALNEDLRSWTAADKAAQITQRKWEAARFAEQRRA
```

7. Select the **NT Variants** tab (RDG Properties), select the NT Variants that you want to add to the reference sequence, then click **Import** to import a tab-delimited variants file or a multi-aligned sequences (.fsta) file.

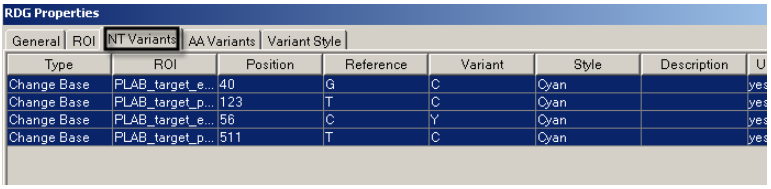
   Note: When importing an amino acid variants file, use a tab-delimited variants format.

8. Click **OK**.

The reference sequence is imported.

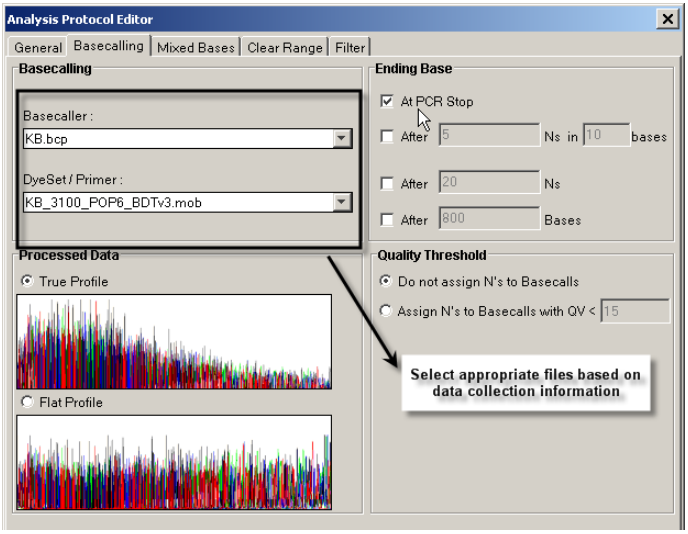


	A	B	C	D	E	F	G
1	Type	ROI	NT position	Reference	Variant	Style	Descri
2	change base	PLAB_target_	40	G	C	Cyan	
3	change base	PLAB_target_	123	T	C	Cyan	
4	change base	PLAB_target_	56	C	Y	Cyan	
5	change base	PLAB_target_	511	T	C	Cyan	

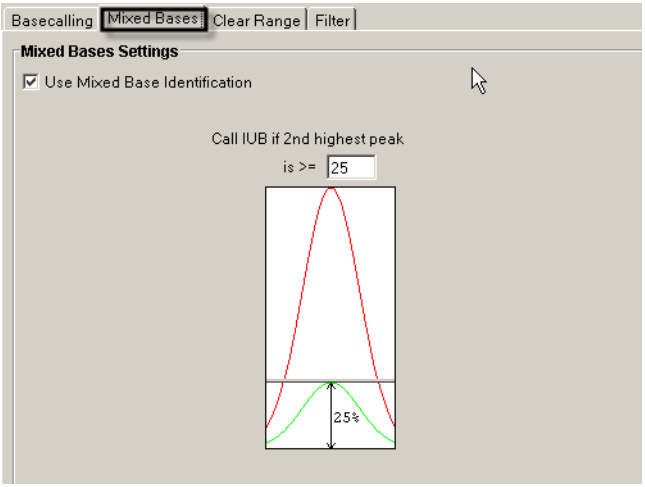


Step 2: Define analysis and display settings

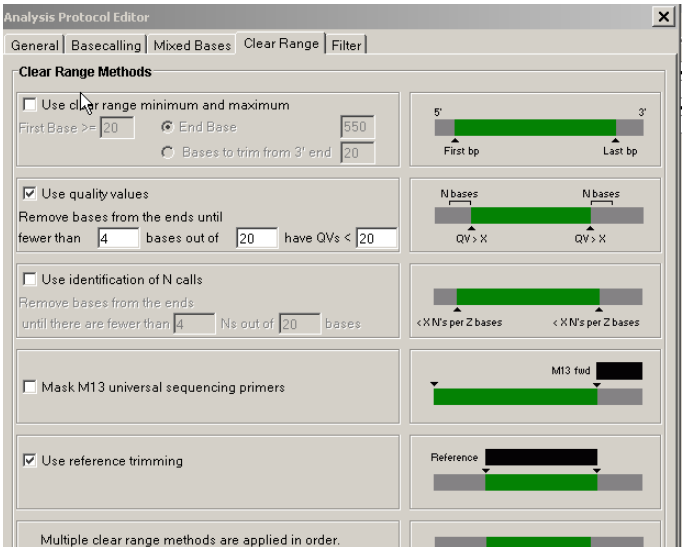
1. In the SeqScape® software, select **Tools ▸ SeqScape Manager**.
2. Select the **Analysis Protocols** tab.
3. Click **New**, then enter a name.
4. Select the Basecalling tab, then select the Basecaller file and Dye/Primer file in the drop-down lists.
5. Unless the project requires a custom setting, keep the Processed Data, Ending Base and Quality Threshold settings at their default values.



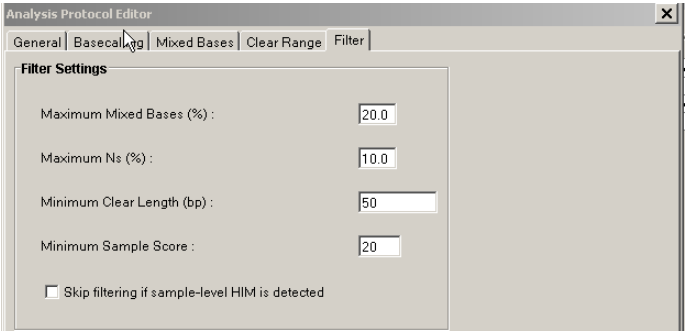
6. In the Mixed Bases tab, specify the secondary peak threshold for mixed base identification.



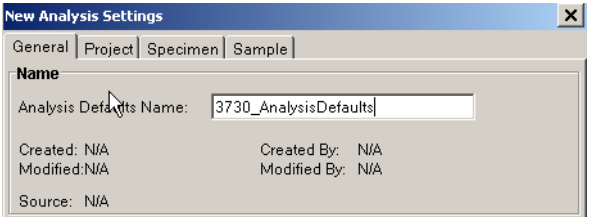
7. In the Clear Range tab, for Use quality values and Use reference trimming, keep the default settings.



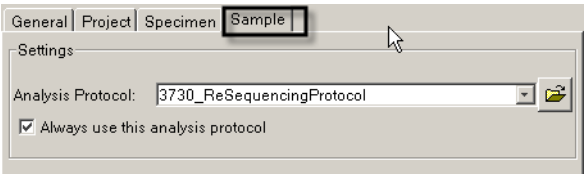
8. In the Filter tab, keep the default Filter Settings, as shown.
9. Click **OK**.
10. In SeqScape Manager, select the **Analysis Defaults** tab.



11. Click **New,** then enter an Analysis Defaults Name.
12. Select the **Sample** tab.



13. In the Analysis Protocol drop-down list, select the analysis protocol that you created.
14. In the **Project** and **Specimen** tabs, keep the default settings.
15. Click **Save**.



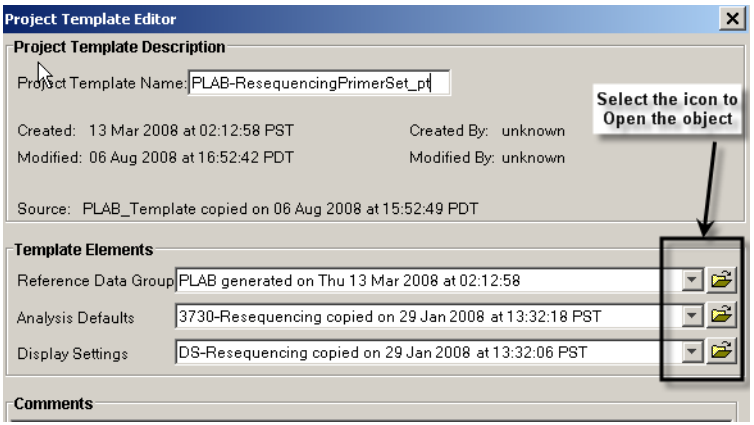
In most cases, you keep the defaults for Display Settings and go to Step 3, “Creating a project template.”

Step 3: Create a project template

To create a project template:

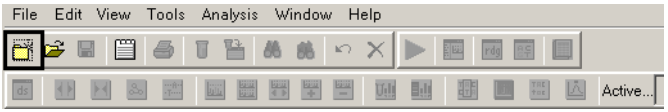
1. In SeqScape® software, select **Tools ▸ SeqScape Manager**.
2. Select the **Project Templates** tab.
3. Click **New**, then enter the Project Template Name.
4. In the Reference Data Group and Analysis Defaults drop-down lists, select the RDG and analysis defaults that you created previously.
5. Keep the default Display Settings.
6. Click **OK**.
7. Close SeqScape Manager.

You now have created the project template.



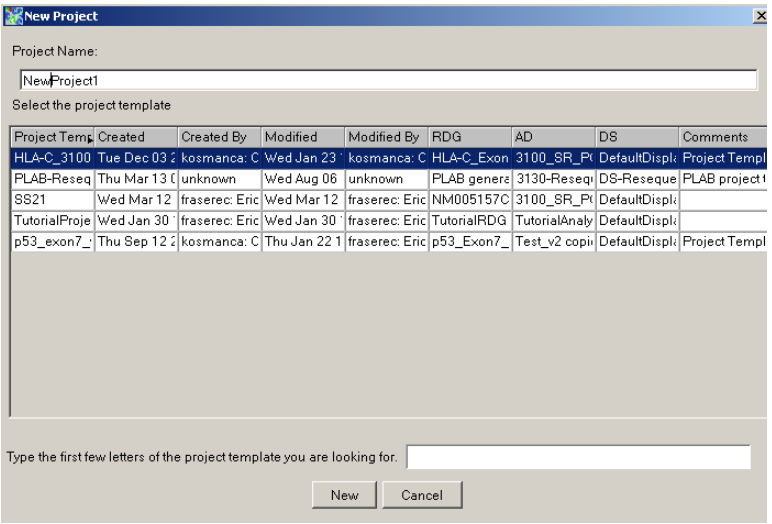
Step 4: Add sample files to the project template

1. In SeqScape® software, select **File ▸ New Project**.



2. Enter a name for the project.
3. In the Project Template list, select the project template that you previously created.
4. Click **New**.
5. To add data to your project, select **File ▸ Import Samples**.

   IMPORTANT! Sample files from the *same* biological source must be grouped together into one specimen.

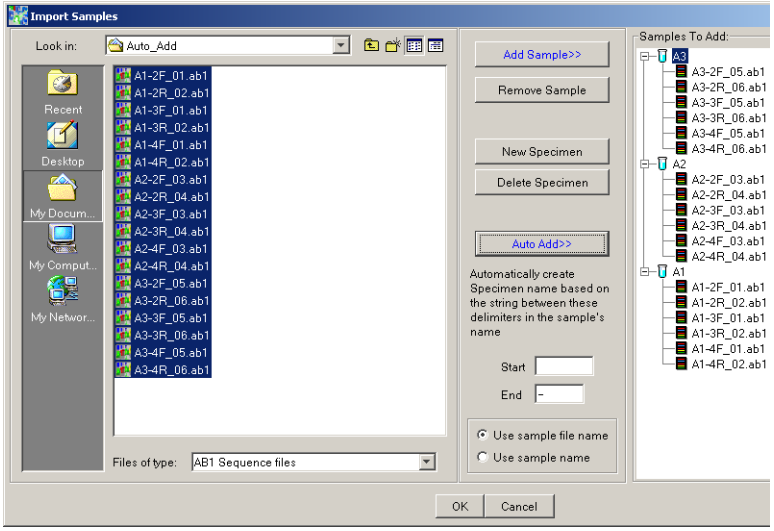


6. Navigate to your data folder, then group your sample files into specimens.
7. For manual specimen creation:
   a. Click **New Specimen**.
   b. Select files belonging to the specimen, then click **Add Sample**.
8. For automatic specimen creation:
   a. Specify the delimiters by assigning alphanumeric values in the Start and End fields.
   b. Select the data folder, then click **Auto Add**.

   Note: A sample-naming convention such as:

   IndividualID_Gene_Exon_WellLoc_F/R.ab1

   is recommended.
9. Click **OK**.



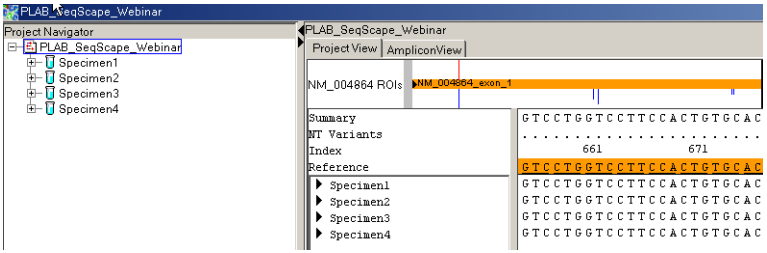
You now have created a project with samples and you are ready to analyze the data.

Step 5: Analyze the data

1. In the SeqScape® software, click (Analyze).

   A progress bar is displayed as the software performs basecalling, trimming, assembly, consensus alignment, and reference comparison on your data.

   After successful analysis, your data appear assembled in the Project view.



Reviewing Data and Results

Reviewing data requires you to:

1. Review data per specimen and assign pass/fail status

2. Review all data in the Project View

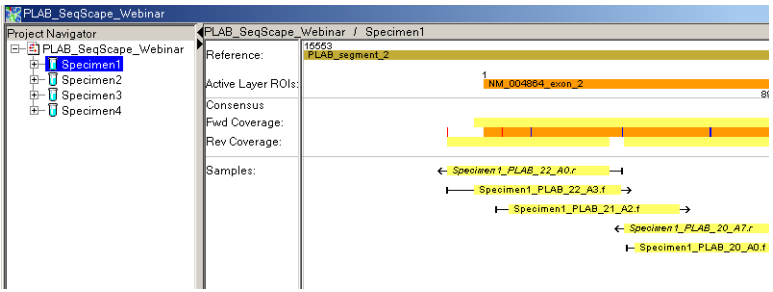
3. View variant results in mutations and AA variants reports

4. View resequencing results in the Genotyping Report

Step 1: Review data per specimen and assign pass/fail status

1. In the Project Navigator (Project view), select a specimen.

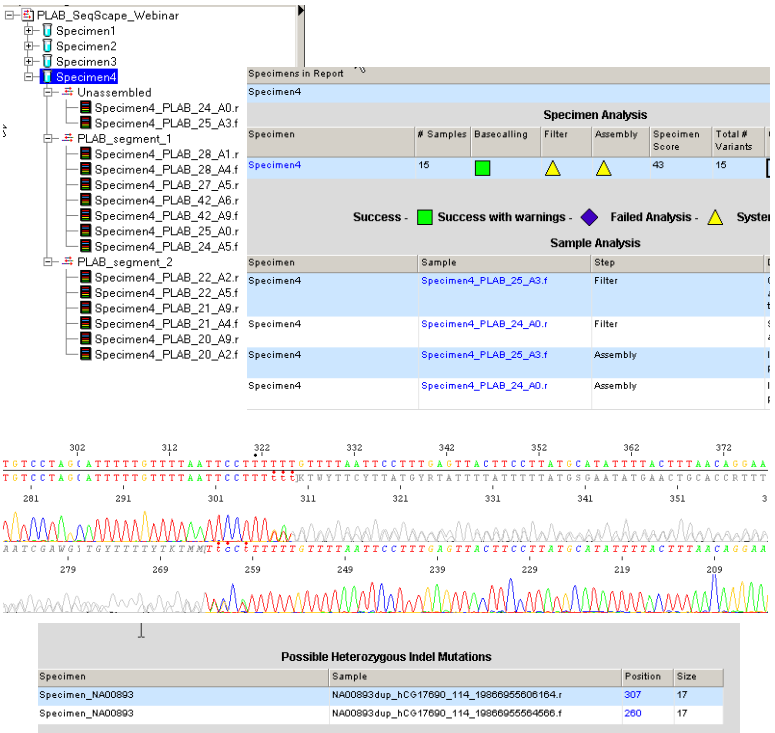
   The specimen view shows the sample distribution within the specimen for the whole reference sequence. Forward and reverse directions are indicated by arrows representing sequence coverage.



2. In the Project Navigator, open the Analysis QC Report to view failed (Unassembled) sample traces.

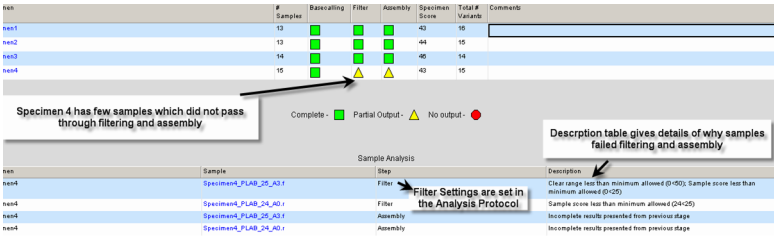
   SeqScape® software automatically filters out low-quality sample traces based on the settings in the Filter tab of the Analysis protocol. The samples are grouped in the unassembled node after analysis. Descriptions of the failed samples appear in the Sample Analysis Table (QC Report).

   The Analysis QC Report displays a list of sample traces with possible heterozygous indel mutations (HIMs). HIMs are detected when two alleles differ by an insertion or deletion in a sample trace.



3. Adjust analysis settings to allow failed samples to assemble in the project (see "Changing Analysis Settings" on page 15 for details).

   Note: You may need to modify your Filter and/or Clear Range analysis settings and then reanalyze data to allow failed samples to pass (Assembled).



4. Select a Segment name, then select the **Assembly** tab to view sample traces that have passed (Assembled).

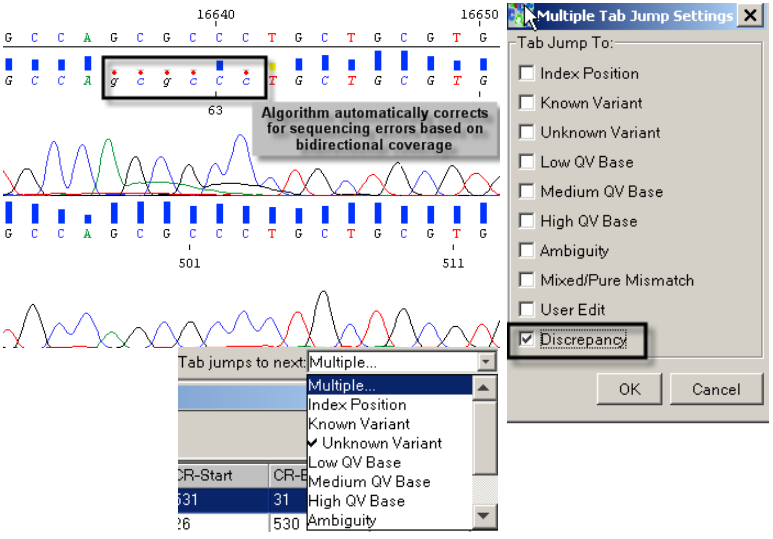
   The Segment Assembly displays all the sample traces in a specimen for a specific region of the reference sequence. To zoom in/out, click **Ctrl +/-**.

5. Edit bases with low quality values, if necessary.

   a. You can customize the bar colors for the quality values (in Display Settings) and flag bases with low quality values in red, for example.

   b. When you switch between the Project view and the Assembly view, the base position is bookmarked for easier editing.

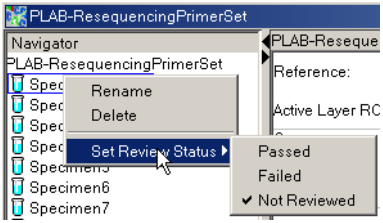
   c. Make sure that **Discrepancy** is selected in Multiple Tab Jump Settings, if required.



The algorithm automatically corrects for sequencing errors by reviewing data quality in both directions (forward and reverse) to arrive at an accurate consensus.

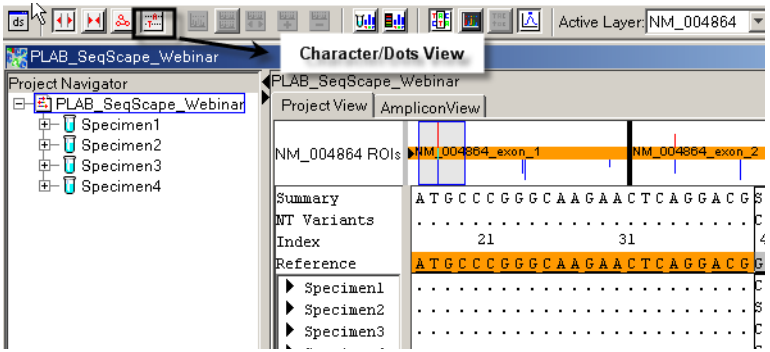
6. Right-click the specimen name to assign Pass or Fail review status.
7. Repeat Steps 1 to 6 for all specimens in the project.

You are now ready to review your data at the project level.

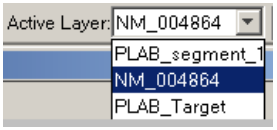


Step 2: Review all data in the Project View

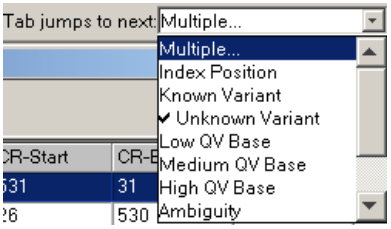
1. Examine variants between the specimen consensus and the reference sequence by one of the following methods:
   a. Select the **Character/ Dots** view. In this view, dots appear for all bases in the reference, and characters appear for variants.



   b. View data by layers. Select a layer in the Active Layer drop-down list.



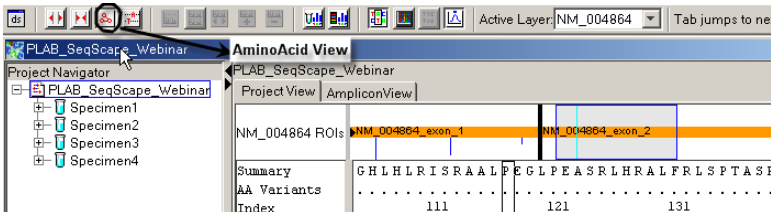
   c. Select **Tab Jumps to next**, then select **Known/ Unknown Variant**.



2. To add a Known Variant, right-click a base, then select **Add Variant**.
3. Click **OK**. The variant is displayed in the Overview pane as a red bar.

4. To view amino acid translation, click **Amino Acid View**.

You are now ready to review the variant results in reports.

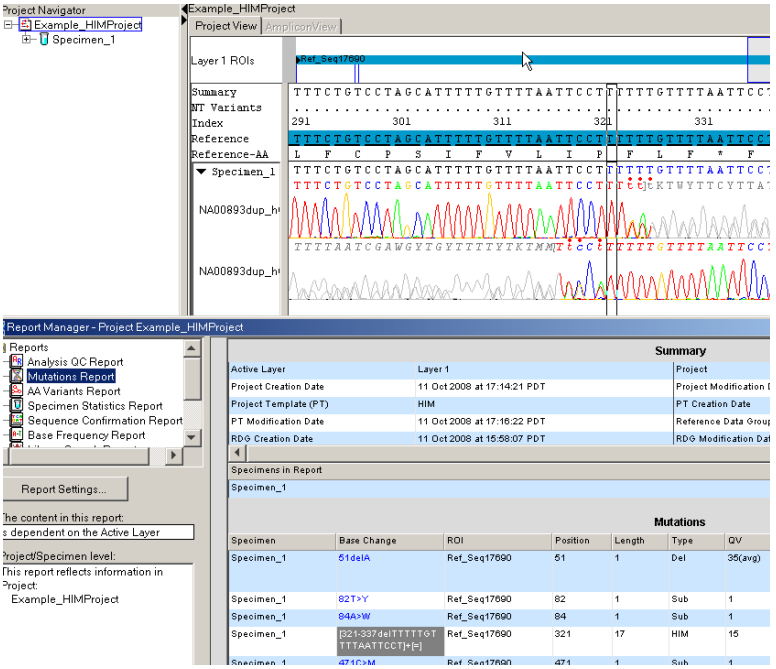


Step 3: View variant results in mutations and AA variants reports

1. In the **Active Layer** drop-down list, select the desired layer, then select the project name in the Project Navigator.

   Note: To view results at the specimen level, select the specimen name.

2. Select **Analysis,** then select **Report Manager**.
3. In the left pane, select the **Mutations Report**.
4. In the right pane, view mutations in the Mutations Table.



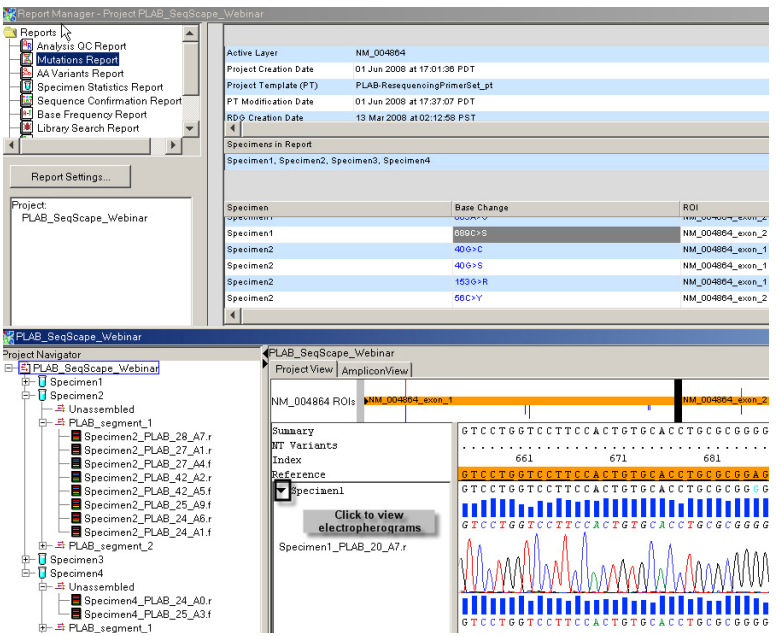
5. In the Mutations Table, click a base change to link the mutation to a specimen consensus in the Project view

   Note: You can review the mutation by observing its quality value and its position in a region of interest (ROI).

   You can arrange mutations by quality value, type, and so on by double-clicking any of the column headers. To remove a column, right-click the column, then deselect the column name.

6. Click the triangle icon next to the specimen name to view the sample electropherogram.

   Look for mutations at the specimen level or “confirmed” mutations due to bidirectional coverage.



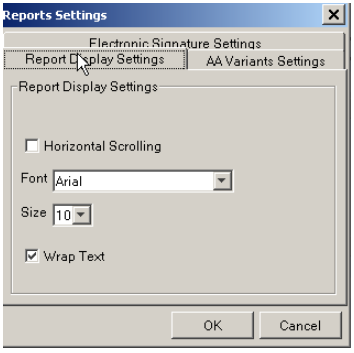
7. Open the Mutations report to view the amino acid effect (AA Change/Effect column) for translated regions.
8. Click any of the amino acids in the AA Change column to open the AA Variants Report.
9. Click an AA Change to link the AA variant to a specimen consensus in the Amino Acid view.

To adjust how the report displays text, click **Report Display Settings**, then deselect **Wrap Text.**

To view reports and data simultaneously, select **Window ▸ Tile** in the main menu.

AA Variants

Specimen	AA Change	Position	Length	Type	Known	NT Change	Description
Specimen1	V14L	14	1	Sub	no	40G>C ROI: NM_004864_exon_1	
Specimen1	S53[T,S]	53	1	Sub	no	157T>W ROI: NM_004864_exon_1	
Specimen1	E326G	326	1	Sub	no	685A>G ROI: NM_004864_exon_2	
Specimen1	D327[D,E]	327	1	Sub	no	689C>S ROI: NM_004864_exon_2	
Specimen2	V14[L,V]	14	1	Sub	no	40G>S ROI: NM_004864_exon_1; 40G>C ROI: NM_004864_exon_1	
Specimen2	E326G	326	1	Sub	no	685A>G ROI:	



Step 4: View resequencing results in the Genotyping Report

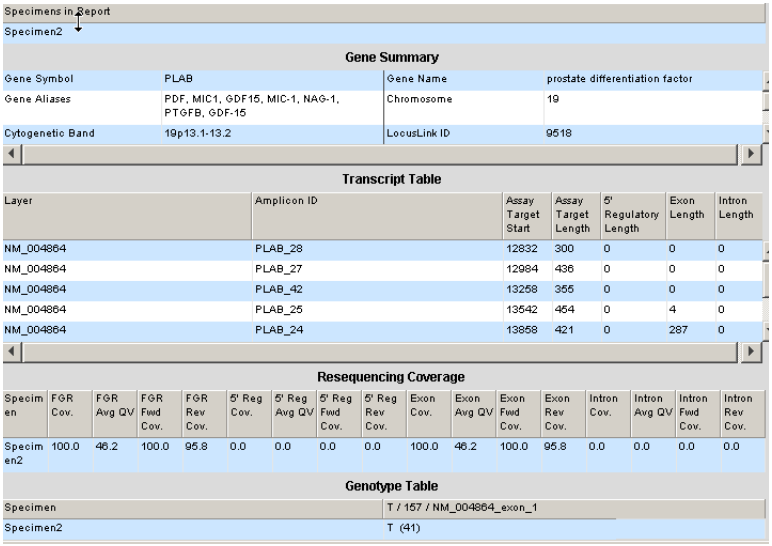
IMPORTANT! The Genotyping Report contains some tables that are filled with the VariantSEQr™ Resequencing System project templates.

1. In the navigation pane, select the project name.

   Note: To view results at the specimen level, select the specimen name.

2. Select **Analysis ▸ Report Manager**.
3. In the left pane, select **Genotyping Report**.
4. In the Resequencing Coverage Table, review the resequencing coverage percentage for each specimen.

   Note: The resequencing coverage is based on the target regions.



5. In the Project view, add genotypes in the Genotyping Report by right-clicking a base, then selecting **Add Genotype**.

   To view the genotypes for each specimen, open the Genotyping Report.

6. Review the genotypes to compare variants or bases for all specimens in a project.

After you complete reviewing your data, if reanalysis is required, go to "Changing Analysis Settings" on page 15. If reanalysis is *not* required, go directly to "Exporting and Printing" on page 20.

Changing Analysis Settings

After reviewing your analysis results, you can make changes to your analyzed project by changing the project settings (analysis, reference, or display).

Changing settings requires you to:

1. Change analysis settings in a project

2. Change reference settings in the project

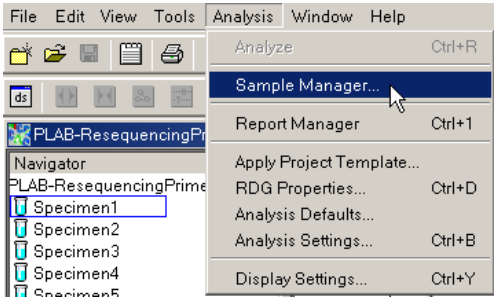
3. Change display settings in the project

4. Apply a project template to an existing project

Step 1: Change analysis settings in a project

To change analysis settings for *all* samples in a project or specimen:

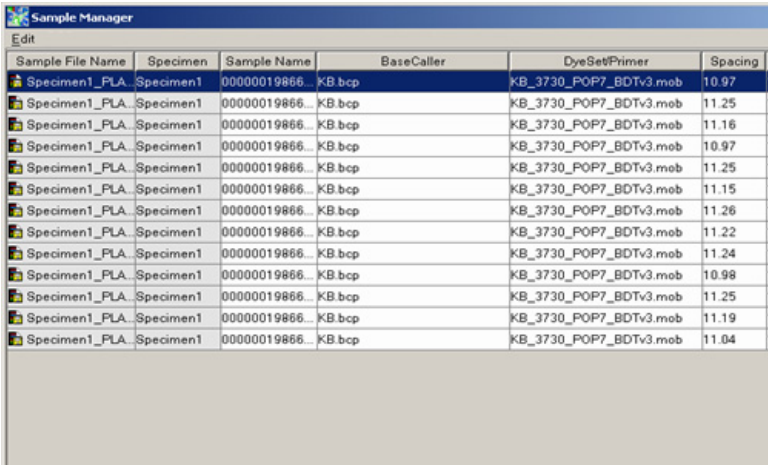
1. In the project navigator, select **Project** or **Specimen Name**.
2. Select **Analysis ▸ Sample Manager**.
3. To change the Basecaller and Dye Set/Primer file, select the desired settings, then click **Apply**.



4. Click **OK** to close the Sample Manager.

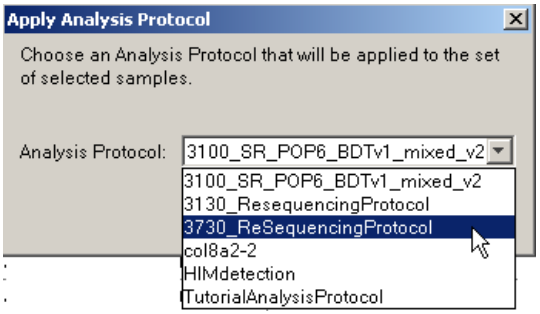
   Note: In the project, a red slash through the files that you changed indicates that the samples are ready to be reanalyzed.

5. Click (Analyze) to analyze the specimen.



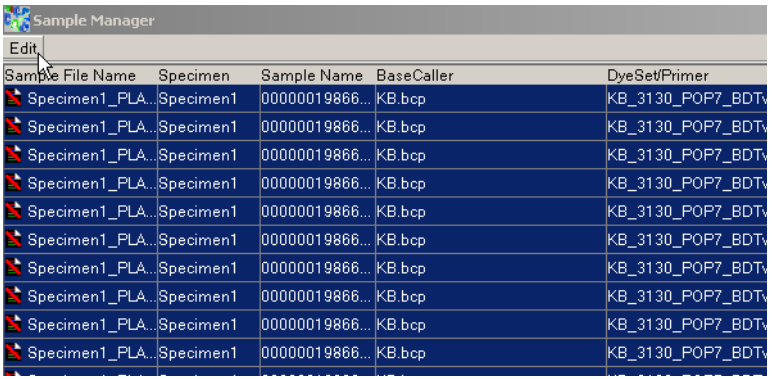
To change *more* than the Basecaller and Dye Set/Primer File:

1. Create a new Analysis protocol in the SeqScape Manager. (See Step 2 on Page 4).
2. Select the desired samples in the Sample Manager, then click **Apply** to apply the Analysis Protocol.



3. Click **OK** to close the Sample Manager.

   Note: In the project, a red slash through the specimen/ files that you changed indicates that the samples are ready to be reanalyzed.



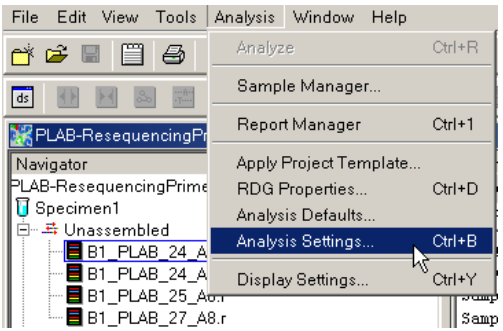
4. Click ▶ (Analyze) to reanalyze the data.

To change the analysis settings in a project for only *one* sample:

5. Select the sample in the Project Navigator.
6. Select **Analysis ▸ Analysis Settings**, then change the settings.

   IMPORTANT! The change applies only to the selected sample and does not change the analysis protocol that was created in the SeqScape Manager.

   Note: A red slash through the selected sample indicates the sample is ready to be analyzed.

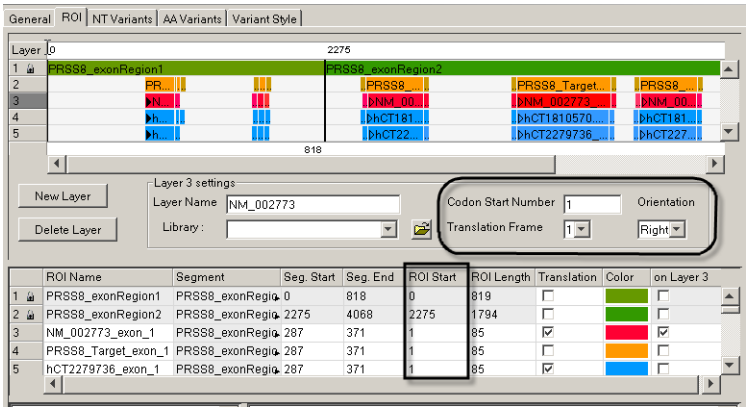


7. Click ▶ (Analyze) to reanalyze the data.

Step 2: Change reference settings in the project

1. Click **rdg** in the project view.
2. Select the **ROI** tab.
3. Change the reference settings within a project by:
   - Changing the Codon Start Number for amino acid translation
   - Changing the translation frames
   - Changing the ROI Start numbering in the ROI pane
   - Adding variants and changing variant styles or colors

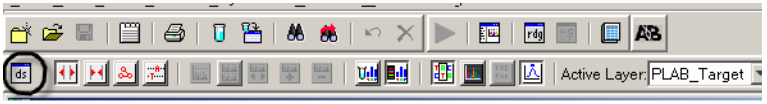
   Note: These changes are saved only in the *existing* project. They do not change in the original reference data group that you created in the SeqScape Manager.

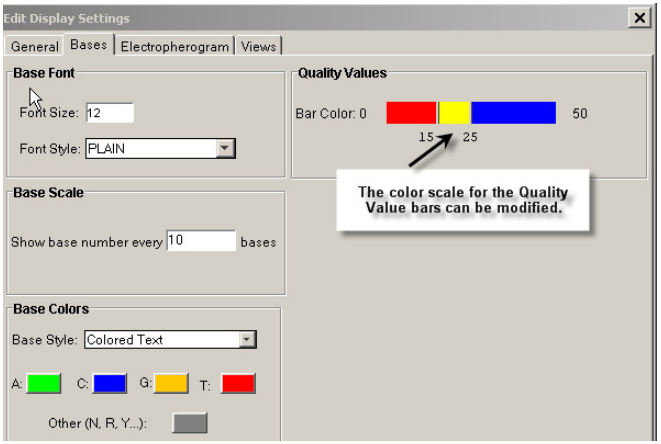


Step 3: Change display settings in the project

To modify the Display Settings within a project:

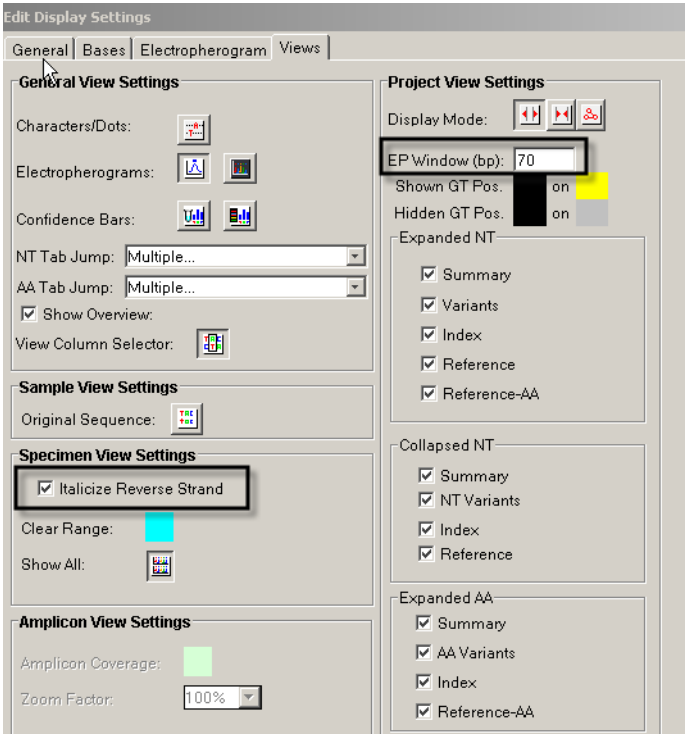
1. In the Project view, click **ds** (Display Settings) to change viewing options.
2. Select the **Bases** tab.
3. Change the color scale as desired to change the quality value bars.
4. Select the **Views** tab.





5. Increase the number of bases that you want to display in the Project view, as indicated in the EP Window field.
6. (Optional) Select **Italicize Reverse Strand**.

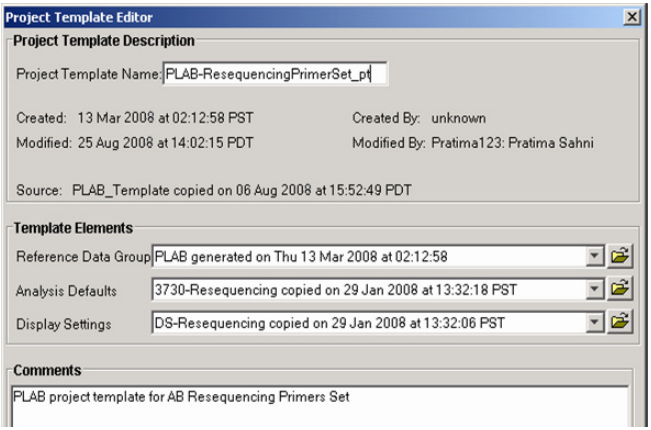
   By default, the display settings show 70 bases and italicize reverse strands.



Step 4: Apply a project template to an existing project

To change an existing project template:

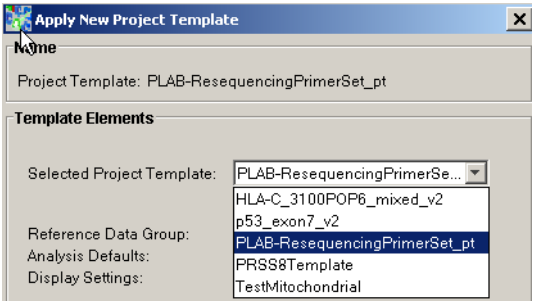
1. Select **Tools ▸ SeqScape Manager,** or click **Tooltip**.
2. Change the data objects in SeqScape Manager, then reselect the sample data objects in the project template to apply the changes.
3. Click **Open Folders** to verify your changes.
4. Click **OK** to close the Project Template editor.



To apply the changed project template to a project:

5. Open the project.
6. Select **Analysis ▸ Apply Project Template**.
7. Select the appropriate Project Template in the drop-down list.
8. Click (Analyze) to reanalyze the data with the modified template.

   Note: All previous edits are lost.



Exporting and Printing

Exporting and printing your analysis results requires you to:

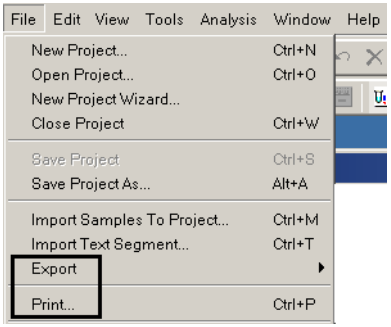
1. Select export options for an analyzed project

2. Set print options at the project, specimen, and segment levels

To determine which printing and exporting options are available in your SeqScape® software, select one of the following in the project navigator:

- Project
- Specimen
- Reference Segment
- Sample

Then select either **File ▸ Export**, or **File ▸ Print.**



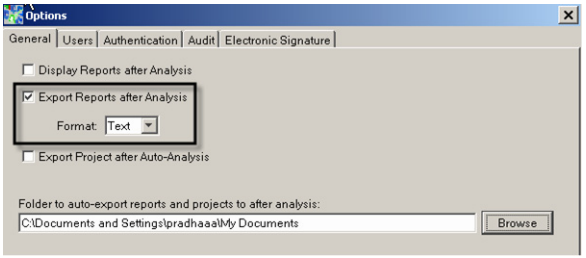
Step 1: Select export options for an analyzed project

To export a selected report from a project:

1. Open the project.
2. Select **Analysis ▸ Report Manager**.
3. Select **Export ▸ Report**.
4. When the Export window opens, select the format tab-delimited text (Microsoft Excel®), PDF, HTML, or XML.

To export all reports automatically after analysis:

5. Select **Tools ▸ Options**.
6. Select **Export Reports After Analysis**, then in the Format drop-down list, select a format to export.



7. Browse to, then select, a target directory to receive the file.
8. Click **OK**.

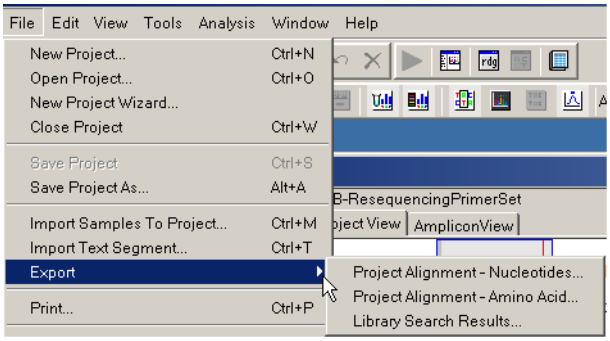
   Note: After a project is reanalyzed, the reports are automatically exported to the specified directory.

To export alignment files:

9. Select the desired project in the project navigator, then select **File ▸ Export**. You can export the Nucleotides project alignment or the Amino Acid project alignment.

   Note: The reference sequence and the specimen consensus sequences are also exported.

10. (Optional) Select **File ▸ Export** for the Specimen level/ Reference Segment level to export the consensus sequence or the aligned sample sequence.
11. (Optional) Select **Electropherogram** to export the Sample Sequence.



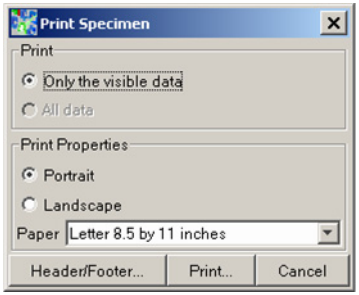
Step 2: Set print options at the project, specimen, and segment levels

Select **All Data** to print the specimen consensus sequences and the reference sequence, but not the Electropherogram data.

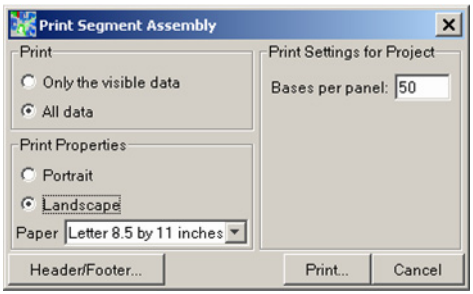
Select **Only the Visible Data** to print only visible data. Only the *displayed* electropherogram data (specimens) are printed.

Note: The electropherogram data for the full project are *not* printed. Only the data that are displayed in the Project window are printed.

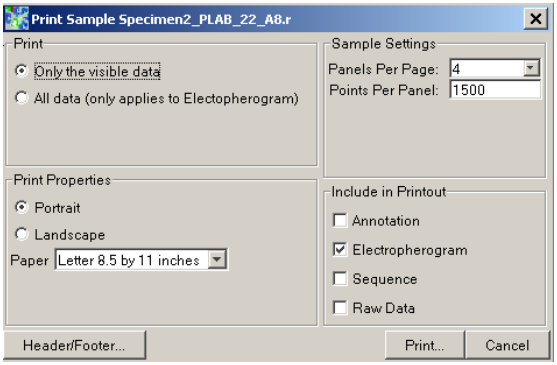
In the Print Specimen view, you can print only the data that are displayed.



In the Print Segment Assembly view, you can print all data *and* the visible data that are displayed.



By selecting a sample you can print the sample electropherogram.



For more information on using SeqScape® software, refer to the *SeqScape® Software User Guide*.

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