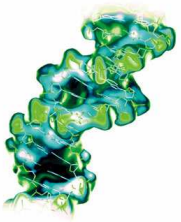


SeqScape Software v2.5

Quick Reference Guide

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.

This Quick Reference Guide describes the four workflows performed in these types of studies:

Workflow for Creating a Project

All analysis in SeqScape software occurs in a project. Before you can create a project you must create a project template that contains:

- A Reference Data Group (RDG)
- Analysis Defaults
- Display Settings

Once a project is created, the project template can be used recurrently.

Note: VariantSEQr customers should proceed directly to Step 4 "Add Sample Files" on Page 7.

Creating a project requires that you follow these steps:

Creating a Project
1. Import the Reference Sequence
2. Define Analysis and Display Settings
3. Create a Project Template
4. Add Sample Files
5. Analyze the Data

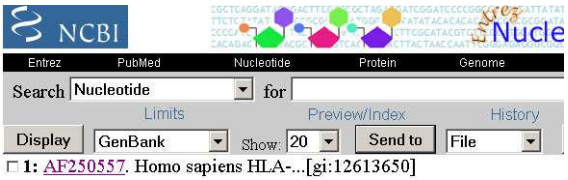
Step 1: Import a Reference Sequence

IMPORTANT! The Reference Sequence format can be any of the following:

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

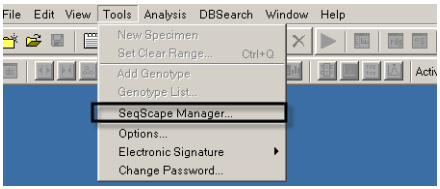
The workflow described below uses a Genbank file for your reference data. To begin, first download a Genbank file, then create a known variants table.

Note: If you download a Genbank file in the NCBI web interface, select **Display as Genbank,** then **Send to File** to save it as a text file that can be imported.

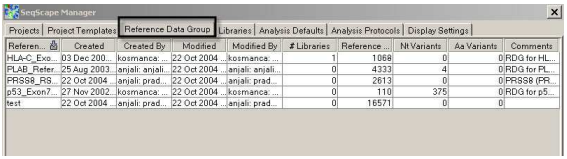


To use a Genbank file as your reference:

1. Open the **SeqScape Manager** from the Tools menu.



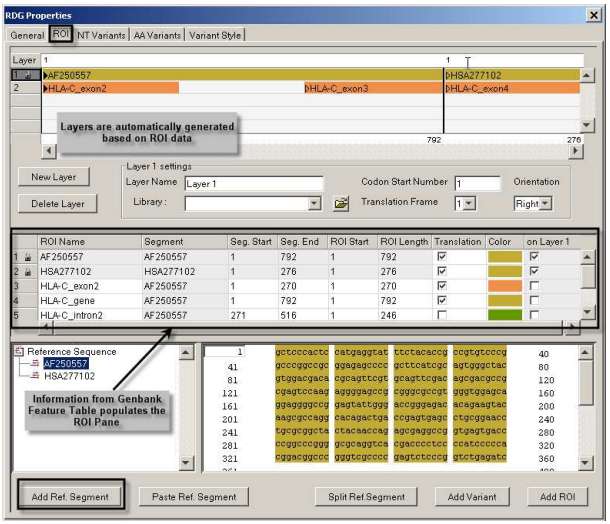
2. Select the **Reference Data Group** tab.
3. Click **New**, then enter a name.



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 on the **Add Ref. Segment** button.



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

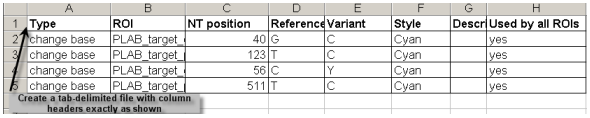
   Note: When the Genbank file imports, information from the feature table populates the Regions of Interest (ROI) table in the SeqScape software and layers are automatically created.

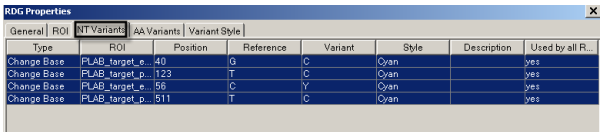
```
FEATURES             Location/Qualifiers
     source          1..792
                     /organism="Homo sapiens"
                     /db_xref="taxon:9606"
                     /map="6p21.3"
     gene            <1..>792
                     /gene="HLA-C"
                     /allele="HLA-Cw*0802"
     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="SHSMRYFYTAVSRPGRGEPRFIAVGYVDDTQFVQFDSDAASPRG
                     EPRAPWVEQEGPEYWDRETQKYKRQAQTDRVSLRNLRGYYNQSEAGSHTLQRMYGCDL
                     GPDGRLLRGYNQFAYDGKDYIALNEDLRSWTAADKAAQITQRKWEAAREAEQRRAYLE
                     GTCVEWLRRYLENGKKTLQRA"
     exon            1..270
                     /gene="HLA-C"
                     /number=2
     exon            517..792
                     /gene="HLA-C"
                     /number=3
```

7. Select the **NT Variants** tab (RDG Properties) and select the NT Variants 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 AA variants file, use a tab delimited variants format.

8. Click **OK**.



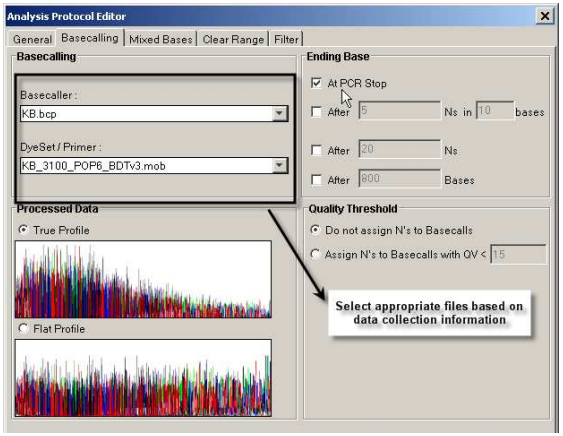


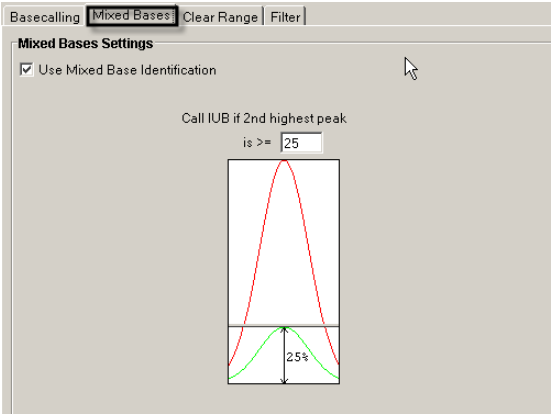
You have now completed importing the reference sequence.

Step 2: Define Analysis and Display Settings

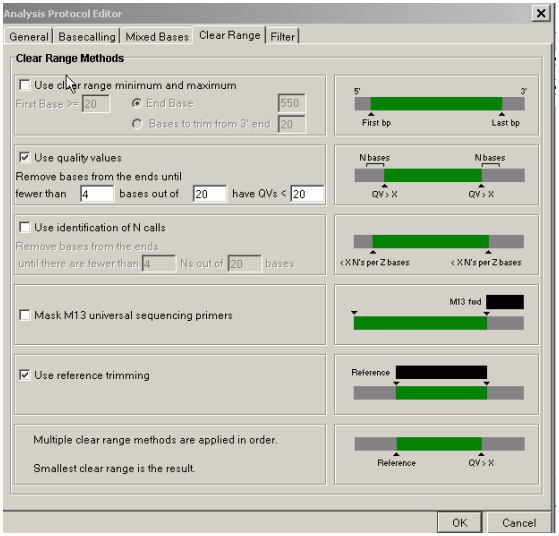
To define analysis defaults:

1. 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 from the drop-down lists.
5. Keep the default settings for Processed Data, Ending Base and Quality Threshold options unless a custom setting is required.
6. Select the **Mixed Bases** tab to define the secondary peak threshold for mixed base identification.



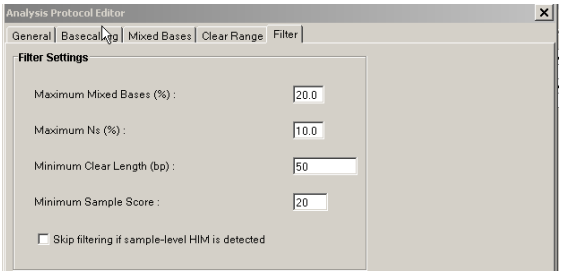


7. Select the **Clear Range** tab.
8. Keep the default settings for Quality Value and Reference Trimming.

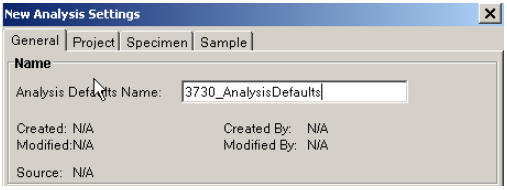


9. Select the **Filter** tab.

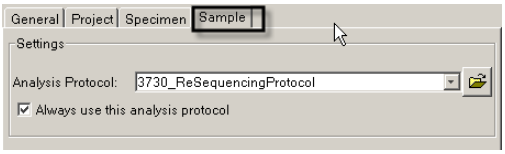
   Keep the default Filter settings as shown.
10. Click **OK**.
11. Select the **Analysis Defaults** tab in the SeqScape Manager.



12. Click **New,** then enter an Analysis Defaults name.
13. Select the **Sample** tab.



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

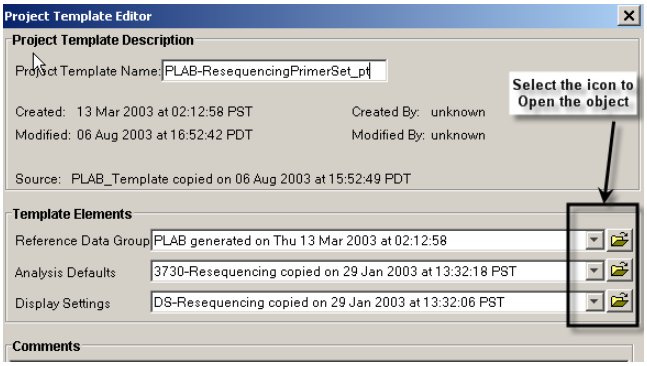


Generally you will keep the defaults for **Display Settings** and proceed to Step 3, “Creating a Project Template.”

Step 3: Create a Project Template

To create a project template:

1. Select **Tools** > **SeqScape Manager**.
2. Select the **Project Templates** tab.
3. Click **New**, then enter a Project Templates name.
4. In the drop-down lists, select the Reference Data Group and Analysis Defaults that you previously created in Steps 1 and 2.
5. Keep the default Display Settings.
6. Click **OK**.
7. Close the SeqScape Manager.



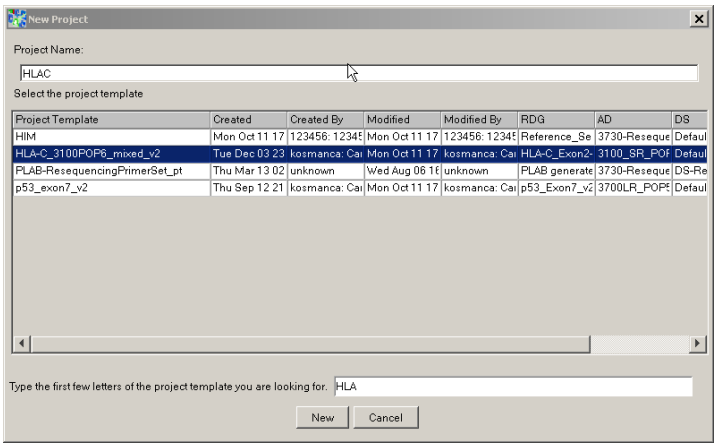
You now have created the Project Template.

Step 4: Add Sample Files

To create a project:

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 you previously created (in Step 3).
4. Click **New**.



5. To add data to your project, select **File** > **Import Samples**.

   IMPORTANT! Groups of sample files from the ***same*** biological source must be grouped together (into one specimen) in SeqScape software.

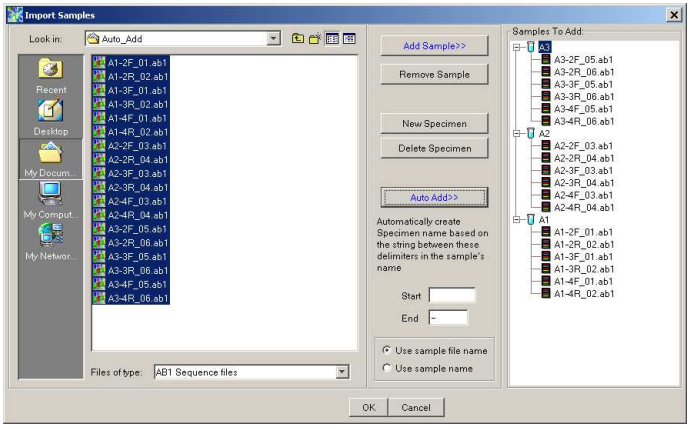
6. Navigate to your data folder, then group your sample files into specimens.
7. For manual specimen creation:
   a. Click **New Specimen**.
   b. Select files belonging to the specimen, then click **Add Samples**.
8. For automatic specimen creation:
   a. Define the delimiters by assigning alphanumeric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

   Note: It is recommended that you use a sample naming convention such as:

   IndividualID_Gene_Exon_ WellLoc_F/R.ab1

9. Click **OK**.

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



Step 5: Analyze the Data

To analyze your data:

1. Click (Analyze) for analysis to begin.

   Note: A progress bar indicates that the software is performing basecalling, trimming, assembly, consensi alignment and reference comparison on your data.

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

Workflow for Reviewing Data

Reviewing data require that you follow these steps:

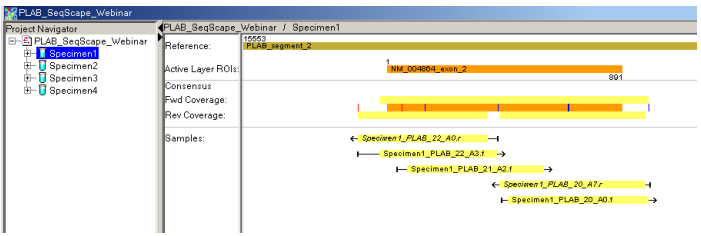
Reviewing Data and Results
1. Review Data per Specimen and Assign Pass/Fail Status
2. Review All Data in Project View
3. View Variant Results in Mutations/AA Variants Reports
4. View Resequencing Results in the Genotyping Report

Step 1: Review Data per Specimen and Assign Pass/Fail Status

To review data by specimen:

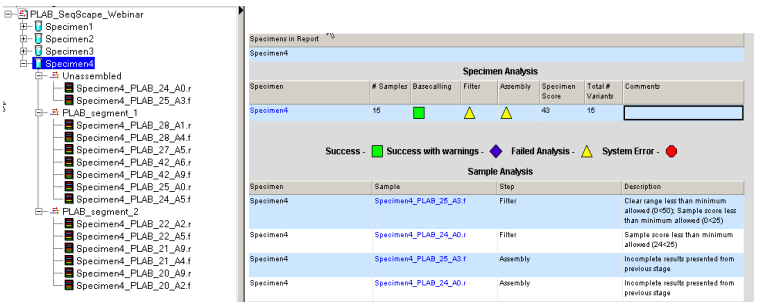
1. In the Project Navigator (Project view), select a specimen.

   Note: The specimen view shows the sample distribution within the specimen across the reference sequence. Forward and reverse orientations are indicated by arrows representing sequence coverage.

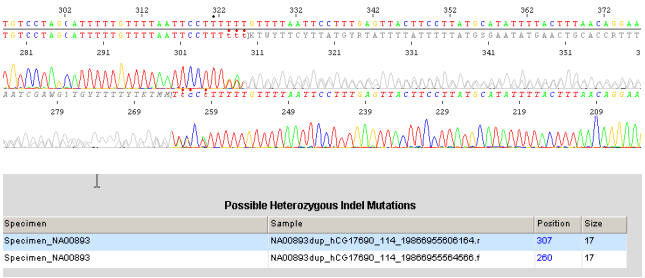


2. In the Project Navigator, open the Analysis QC Report to view failed sample traces (Unassembled).

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



Note: The Analysis QC Report provides 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.

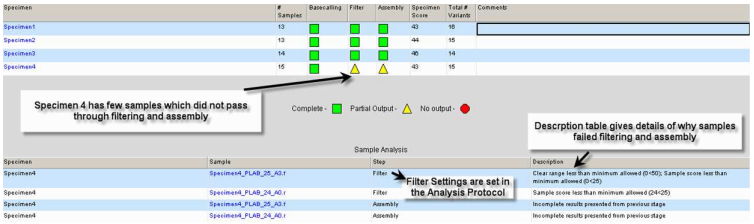


Possible Heterozygous Indel Mutations

Specimen	Sample	Position	Size
Specimen_NA00893	NA00893dup_hCG17690_114_19866955606164.r	307	17
Specimen_NA00893	NA00893dup_hCG17690_114_19866955564666.f	260	17

3. Adjust analysis settings to allow failed samples to assemble in the project (see Modifying Settings Workflow for more detail).

   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).

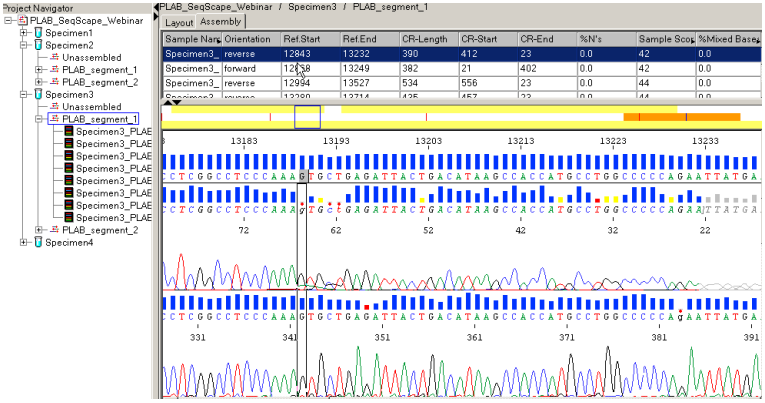


4. Select a Segment name, then select the **Assembly** tab to view passed sample traces (Assembled).

   Note: The Segment Assembly displays the assembly of sample traces within a specimen for a specific region of the reference sequence. To zoom in/out, select **Cntl +/-**.

5. Edit bases with low quality values if necessary.

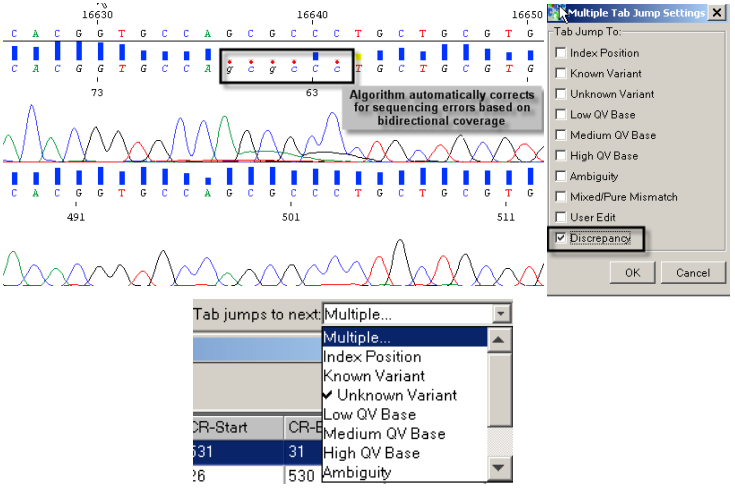
   a. You can customize the bar colors for the quality values (in Display Settings) and flag bases with low quality values in red, for example.



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

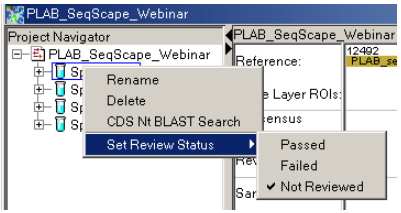
c. Make sure **Discrepancy** is checked in Multiple Tab Jump Settings, if required.

Note: 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/Fail review status.
7. Repeat Steps 1 to 6 for all specimens in the project.

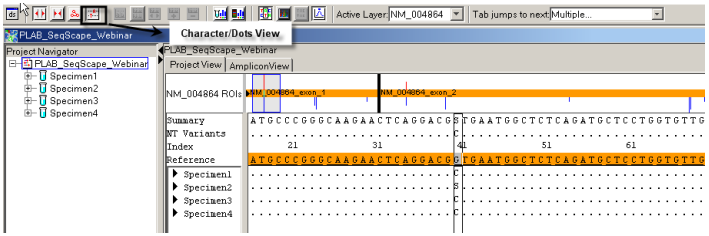
You are now ready to review your data at the project level.



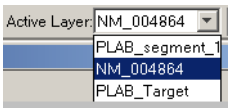
Step 2: Review all Data in Project View

To review all data:

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



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



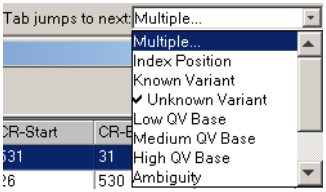
c. Select the **Tab Jump to Next** field. In this setting, you can select **Known/ Unknown Variant**.

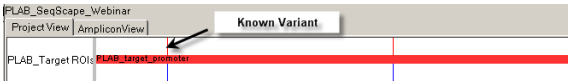
2. To add a Known Variant right-click a base, then select **Add Variant**.

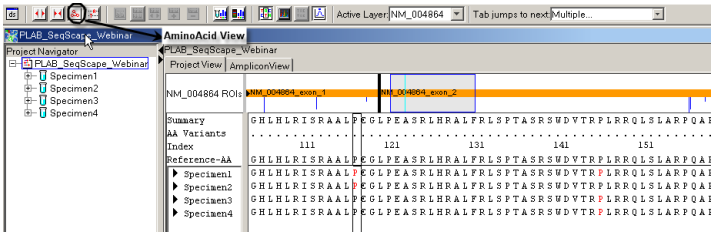
3. Click **OK**. The variant is displayed in the Overview pane as a red bar.

4. To view Amino Acid translation, click on the **Amino Acid** view.

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







Step 3: View Variant Results in Mutations/AA Variants Reports

To view variant results in reports:

1. Select the desired layer using the **Active Layer** drop-down list, and highlight the project name within the Project Navigator.

   Note: To view results at the specimen level, highlight 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 on a base change to link the mutation to a specimen consensus in the Project view.

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

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

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

   Note: Look for mutations at the specimen level or "confirmed" mutations due to bidirectional coverage.

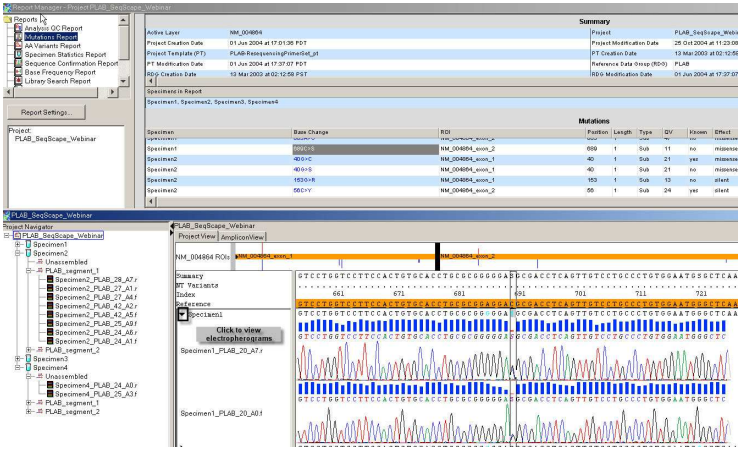
7. Open the Mutations report to view the amino acid effect (AA Change/Effect column) for translated regions.

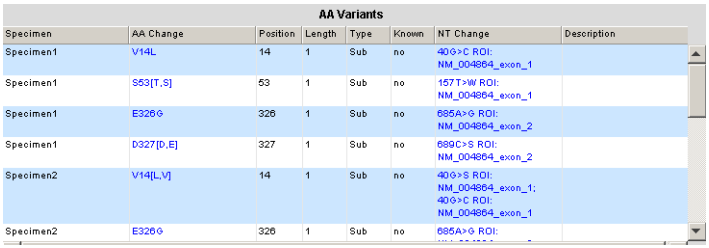
8. Click any of the amino acids in the AA Change column to navigate to the AA Variants Report.

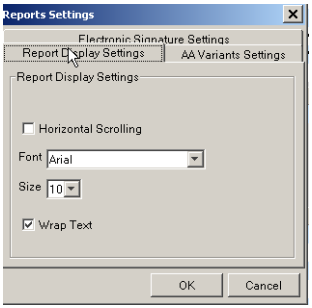
9. Click an AA Change to link the AA variant to a specimen consensus in the Amino Acid view.

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

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







Step 4: View Resequencing Results in the Genotyping Report

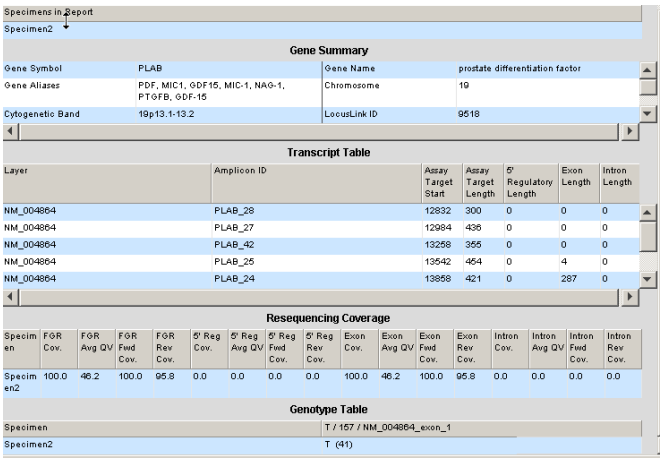
IMPORTANT! The Genotyping Report contains some tables that are filled with the VariantSEQr™ Resequencing System project templates.

To view resequencing results in a report:

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

   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 **Genotyping Report**.



Specimens in Report

Specimen2

Gene Summary

Gene Symbol	PLAB	Gene Name	prostate differentiation factor
Gene Aliases	PDF, MIC1, GDF15, MIC-1, NAG-1, PTGFB, GDF-15	Chromosome	19
Cytogenetic Band	19p13.1-13.2	LocusLink ID	9518

Transcript Table

Layer	Amplicon ID	Assay Target Start	Assay Target Length	5' Regulatory Length	Exon Length	Intron Length
NM_004864	PLAB_28	12832	300	0	0	0
NM_004864	PLAB_27	12984	436	0	0	0
NM_004864	PLAB_42	13258	355	0	0	0
NM_004864	PLAB_25	13542	454	0	4	0
NM_004864	PLAB_24	13858	421	0	287	0

Resequencing Coverage

Specimen	FGR Cov.	FGR Avg QV	FGR Fwd Cov.	FGR Rev Cov.	5' Reg Cov.	5' Reg Avg QV	5' Reg Fwd Cov.	5' Reg Rev Cov.	Exon Cov.	Exon Avg QV	Exon Fwd Cov.	Exon Rev Cov.	Intron Cov.	Intron Avg QV	Intron Fwd Cov.	Intron Rev Cov.
Specimen2	100.0	46.2	100.0	95.8	0.0	0.0	0.0	0.0	100.0	46.2	100.0	95.8	0.0	0.0	0.0	0.0

Genotype Table

Specimen	T / 157 / NM_004864_exon_1
Specimen2	T (41)

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**.

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

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

If you completed reviewing your data, and reanalysis is required, go to “Workflow on Modifying Settings” on the next page. If reanalysis is *not* required, you can go directly to “Workflow on Exporting and Printing.”

Workflow for Modifying Settings

You can make modifications to your analyzed project by changing the settings (analysis, reference or display) after reviewing your analysis results.

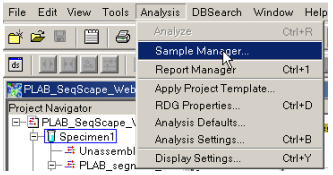
Modifying settings requires that you follow these steps:

Modifying Analysis Settings
1. Modify Analysis Settings in the Project
2. Modify Reference Settings in the Project
3. Modify Display Settings in the Project
4. Apply a Project Template to an Existing Project

Step 1: Modify Analysis Setting in the Project

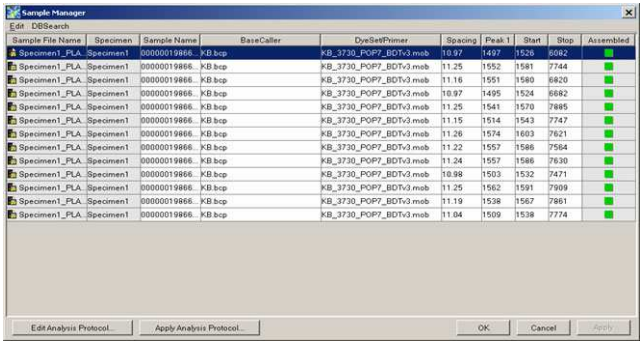
To modify analysis settings for ***all*** samples in a Project or Specimen:

1. Select **Project** or **Specimen Name** in the Project Navigator.
2. Select **Analysis** > **Sample Manager**.



3. If you want to adjust 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 adjusted indicates the samples are ready to be reanalyzed.

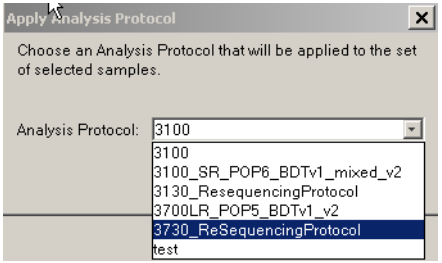


5. Click (Analyze) to analyze the specimen.

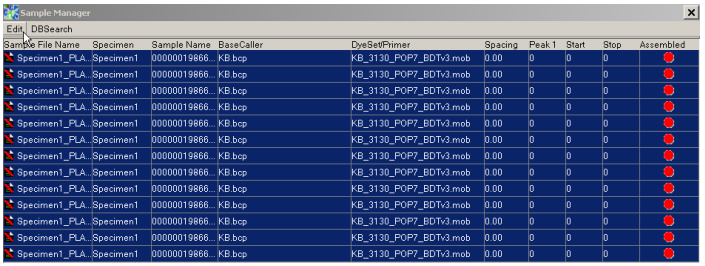
If you need to adjust ***more*** than the Basecaller and Dye Set/Primer File:

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 adjusted indicates that the samples are ready to be reanalyzed.



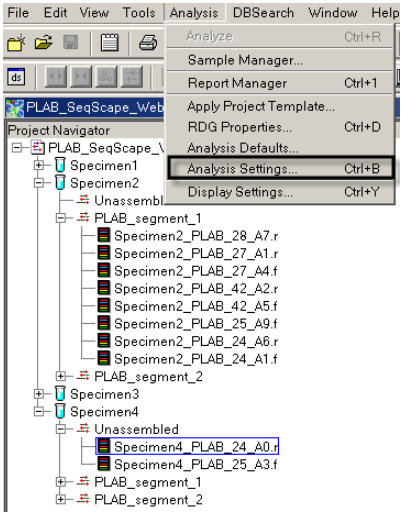
4. Click (Analyze) to reanalyze the data.

If you are adjusting the Analysis Settings in a Project for just ***one*** sample:

1. Select the sample in the Project Navigator.
2. Go to **Analysis** > **Analysis Settings** and make your modifications to the settings.

   IMPORTANT! The change applies only to the selected sample and does not modify the Analysis protocol created in the SeqScape Manager.

   Note: A red slash through the selected sample indicates the sample is ready to be analyzed.
3. Click on the icon to reanalyze the data.

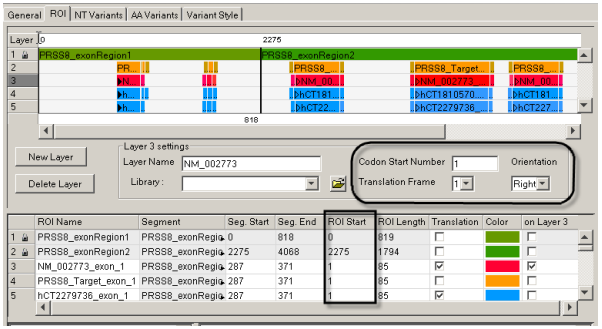


Step 2: Modify Reference Settings in the Project

To make changes such as adding variants to an existing reference:

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

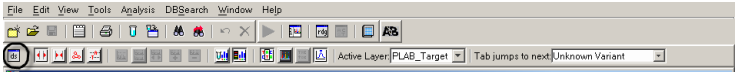
Note: These modifications are saved only within the *existing* project. They do not change in the original Reference Data Group that you created in the SeqScape Manager.

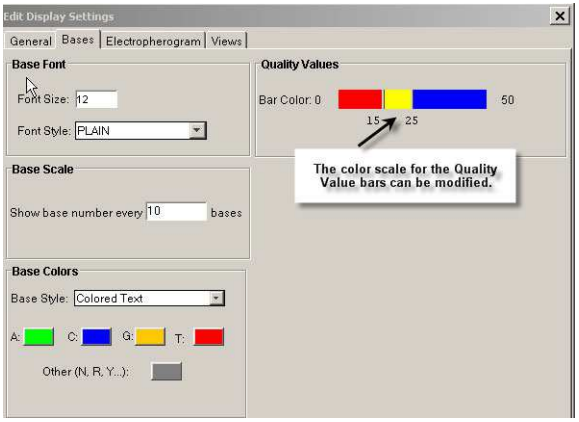


Step 3: Modify Display Settings in the Project

To modify the Display Settings within a project:

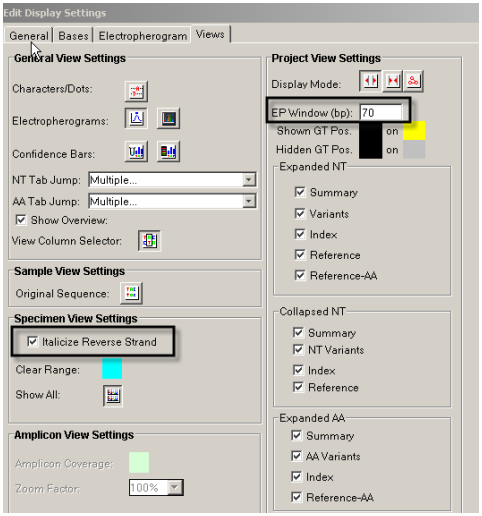
1. In the Project view, click **ds** (Display Settings) to modify viewing options.
2. Select the **Bases** tab.
3. Change the color scale as desired to modify the quality value bars.
4. Select the **Views** tab.





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

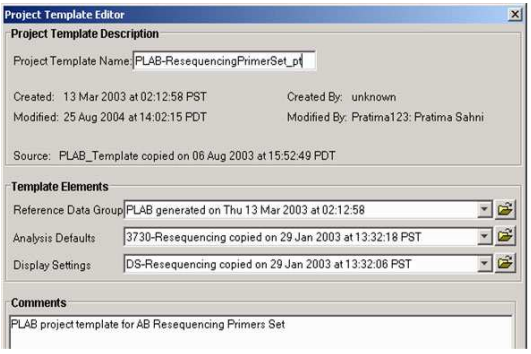
   Note: The display settings show 70 bases and italicize reverse strands by default.



Step 4: Apply a Project Template to an Existing Project

To make changes to an existing Project Template:

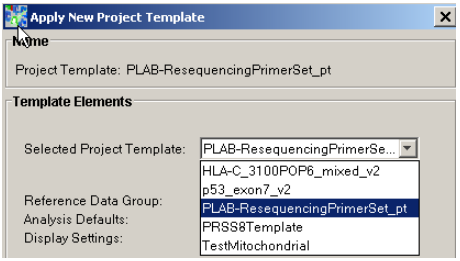
1. Select **Tools** > **SeqScape Manager,** or simply click **Tooltip**.
2. Modify the data objects within SeqScape Manager and re-select the same data objects within the Project Template to apply changes.
3. Click **Open Folders** to verify your modifications.
4. Click **OK** to close the Project Template editor.



To apply the modified Project Template:

1. Open the project.
2. Select **Apply Project Template** (Analysis menu).
3. Select the appropriate Project Template from the drop-down menu.
4. Click (Analyze) to reanalyze the data with the modified template.

   Note: All previous edits are lost.



Workflow for Exporting and Printing

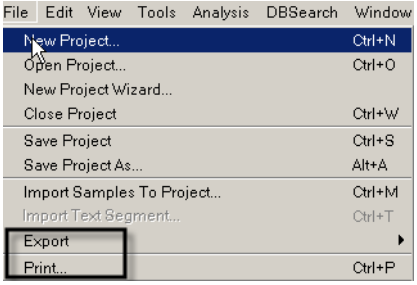
In this workflow we review optional selections for exporting and printing your analysis results.

Exporting and Printing
1. Select Export Options for an Analyzed Project
2. Set Print Options at Project/Specimen/Segment Levels

To determine which printing and exporting options are available in SeqScape, select a level in the Project Navigator from one of the four choices:

- Project
- Specimen
- Reference Segment
- Sample

1. Select **File** > **Export**, **File** > **Print**.



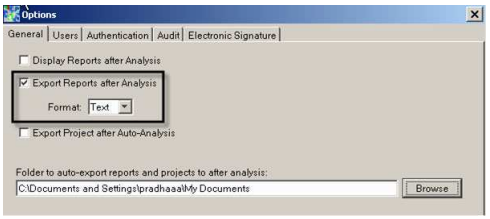
Step 1: Select Export Options for an Analyzed Project

To export a selected report from a Project:

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

To export all reports automatically after analysis:

1. Select **Tools** > **Options**.
2. Select **Export Reports After Analysis**, then in the Format drop-down list, select a format to export.



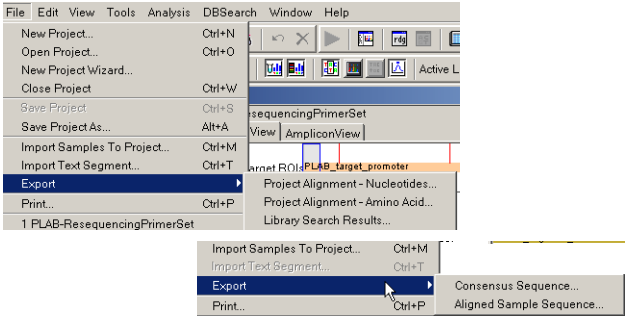
3. Browse to set a target directory for the file.
4. Click **OK**.

   Note: Once a project is reanalyzed, the reports will *automatically* export to the set directory.

To export alignment files:

1. Select the project (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 also export.
2. (Optional) Select **File** > **Export** for the Specimen level/ Reference Segment level to export the consensus sequence or the aligned sample sequence.
3. (Optional) Select **Electropherogram** to export the Sample Sequence.



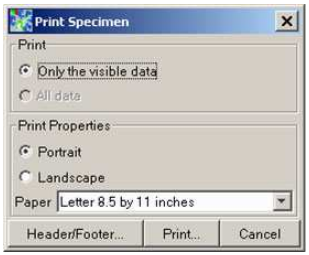
Step 2: Set Print Options at the Project, Specimen, Segment Levels

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

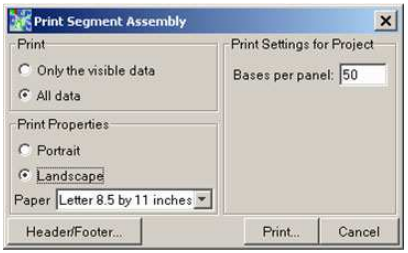
Select **Only the Visible Data** to print only visible data. Consequentially, only the *displayed* electropherogram data (specimens) are printed.

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

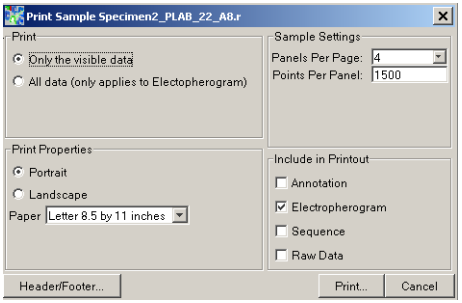
From the Specimen view, you can print only the data that are displayed.



From the 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 v2.5, refer to the *SeqScape Software User Manual*.

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