

# Frequently Asked Questions

## SeqScape® Software Version 2.6

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### SUBJECT: SeqScape® FAQs

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## Upgrading FAQs

There are four available versions of SeqScape® Software. See the *SeqScape Software User Guide* for more information.

Table 1 Upgrading questions and answers

Question	Answer
How does SeqScape software v2.6 differ from v2.1?	Refer to the <i>SeqScape Software User Guide</i> , Chapter 1, "Introduction to SeqScape Software."
<p>What happens to my data when I upgrade my SeqScape software?</p> <p>What happens to my data if I do an install of the SeqScape software v2.6?</p>	<p>If you install the SeqScape software v2.6 upgrade, data from SeqScape software v2.5 is stored in the Data Store backup folder that is named according to the version of SeqScape software previously installed (that is, Data v2.5). All previous contents of the Data Store are copied to the new Data Store.</p> <p>If you purchase an upgrade to SeqScape v2.6 from versions prior to v2.5 (v1.X and V2.0.X, 2.1.X), you must install the full version of SeqScape software v2.6.</p> <p>When you install the full version of SeqScape software v2.6, you must first export the data objects from the SeqScape Manager in the previous version of SeqScape software, then import them into SeqScape software v2.6.</p>
What happens to the data that I created with the demo version v2.5 when I upgrade to the full version of SeqScape software?	<p>Export the data generated with the 30-day demo before it expires, install SeqScape software v2.6, then import the data into the full version of the SeqScape software v2.6.</p> <p>To export data, select the desired object from the SeqScape Manager window, then click <b>Export</b>. To import the object into the full copy of the SeqScape software v2.6, use the SeqScape Manager.</p>

## Training and Documentation FAQs v2.6

Table 2 Training and documentation questions and answers


Question	Answer
How do I train myself on SeqScape software?	The best way to train yourself on SeqScape software is to use the training movie and the software tutorial included in the software package. A printed tutorial is included with all SeqScape software versions except for the demo version. The tutorial is also available online (see below). Additionally, workflows are available for SeqScape software v2.6
Where can I find resources/documentation on SeqScape software?	<p>All documentation for SeqScape software is available on the installation CD. After you install SeqScape software, click <b>Start</b> &gt; <b>Programs</b> &gt; <b>Applied Biosystems</b> &gt; <b>SeqScape</b> to access documents.</p> <p>You can also find documentation about SeqScape software at: <a href="http://www.appliedbiosystems.com">www.appliedbiosystems.com</a> . In the Support tab, click <b>General Literature</b> to access the Products and Services Literature page. Enter SeqScape as the keyword to find literature on using SeqScape software.</p>

## SeqScape Software Basics FAQs

Table 3 SeqScape software basics questions and answers

Question	Answer
What is SeqScape software?	SeqScape software is a resequencing software tool designed to identify nucleotide variants, amino acid variants, and library sequences that match each consensus sequence.
SeqScape software can be used for which sequencing application?	SeqScape software can be used for SNP discovery and validation, mutation analysis and heterozygote identification, sequence confirmation for mutagenesis or clone-construct confirmation studies, and the identification of genotype, allele and haplotype from a library of known sequences.

Table 3 SeqScape software basics questions and answers (*continued*)

Question	Answer
<p>What is the overall workflow for analyzing and reviewing data?</p>	<p>All analysis in SeqScape software occurs in a project. Analysis and review of the data requires that you:</p> <ol style="list-style-type: none"> <li>1. Create analysis defaults and display settings (or use settings previously created).</li> <li>2. Create an RDG (or use one previously created).</li> <li>3. Create a project template (or use one previously created).</li> <li>4. Create and analyze a project by adding sample files to a project template.</li> <li>5. Review the results (view and/or edit the data and view the reports).</li> <li>6. Export or print the results and reports.</li> </ol>
<p>What happens in the SeqScape software when I click  (Analyze)?</p>	<p>Each time you click the Analyze button, the software performs the following operations on specimens that have not been analyzed:</p> <ol style="list-style-type: none"> <li>1. Basecalls and determines quality values.</li> <li>2. Identifies mixed bases. (This step can be bypassed if data was previously basecalled.)</li> <li>3. Trims low-quality sequence ends.</li> <li>4. Filters (omit poor-quality sequences).</li> <li>5. Assembles sequences to the reference.</li> <li>6. Generates a consensus sequence for each specimen.</li> <li>7. Aligns each consensus sequence to the reference sequence.</li> <li>8. Compares each consensus to the reference.</li> <li>9. Searches the allele Library for matches to each consensus sequence. (This step can be bypassed if you do not need to identify allele matches.)</li> <li>10. Generates reports.</li> </ol>

# General SeqScape Software FAQs

Table 4 General questions and answers

Question	Answer
<p><b>Instruments</b> – What Applied Biosystems instruments can I use to generate data for SeqScape software?</p>	<p>You can use ABI PRISM® 310, 3100, 3100-<i>Avant</i>, 3700, and Applied Biosystems 3130/3130<i>xl</i> and 3730/3730<i>xl</i> instruments.</p> <p><b>Note:</b> The software also accepts text sequences in FASTA format.</p>
<p><b>Instruments</b> – Can SeqScape software be used to analyze data that was generated on instruments other than Applied Biosystems instruments?</p>	<p>No. Sequencing data generated on platforms other than Applied Biosystems platforms are not compatible with SeqScape software.</p>
<p><b>Sample files</b> – Can SeqScape software be used for autoanalysis of sample files?</p>	<p>Yes. SeqScape software v2.6 can be used to autoanalyze sample files from Applied Biosystems 3130/3130<i>xl</i> and 3730/3730<i>xl</i> instruments and the ABI PRISM® 310, 3100/3100-<i>Avant</i> Genetic Analyzers. For information on how to configure the software for the autoanalysis of sample files, see the <i>SeqScape Software User Guide</i>, Appendix B, “Automating Analysis.”</p>
<p><b>Sample files</b> – What instrument sample files can I use with the KB basecaller?</p>	<p>You can use the KB™ basecaller in SeqScape software to analyze sequencing sample files generated from the ABI PRISM® 310, 3100/3100-<i>Avant</i> Genetic Analyzers and Applied Biosystems 3130/3130<i>xl</i> and 3730/3730<i>xl</i> instruments.</p> <p>KB Basecaller v1.4 is integrated with SeqScape Software v2.6. For details, please check <i>KB basecaller v1.4 FAQ</i> provided with SeqScape software v2.6</p>
<p>How can I share my work with someone at a different site? What should I send them?</p>	<p>All sample files, analysis parameters, reference sequence, and analysis results are saved in every SeqScape software project file. You can share these files with anyone who has the software by exporting the project or data objects, then importing them into their software on their computer. There is no link between the SeqScape software installed on different computers.</p> <p>You can also share project templates, which contain the reference sequence and analysis parameters. Colleagues 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.</p>

Table 4 General questions and answers (continued)

Question	Answer
<p><b>Files</b> – What are the file types/formats accepted by SeqScape software?</p>	<p><b>Reference Sequences</b> – SeqScape software accepts the following file formats for reference sequences:</p> <ul style="list-style-type: none"> <li>• Genbank File Format (with a .fcgi, .cgi, or .gb extension)</li> <li>• .txt (text) file format</li> <li>• .ab1 file format</li> <li>• .fasta (FASTA) file format</li> <li>• .seq file format</li> <li>• Aligned sequences in .fasta (FASTA) format<sup>a</sup></li> </ul> <p><b>Data Sequences</b> – SeqScape software accepts the following file formats for data sequences used for analysis:</p> <ul style="list-style-type: none"> <li>• .ab1 files (previously basecalled or not basecalled)</li> <li>• .txt (TEXT) files</li> <li>• .fasta (FASTA) files</li> <li>• .seq files</li> </ul> <p><b>Nucleotide Variants</b> – SeqScape software accepts the following file formats for nucleotide variants:</p> <ul style="list-style-type: none"> <li>• .fasta file containing a set of aligned sequences in FASTA format.</li> <li>• Tab-delimited text (.txt) file that lists one variant per line and eight column headings: Type, ROI, NT position, Reference, Variant, Style, Description, and Used by all ROIs.</li> </ul> <p><b>Amino Acid Variants</b> – SeqScape software accepts the following file formats for amino acid variants:</p> <ul style="list-style-type: none"> <li>• Tab-delimited text (.txt) file that lists one variant per line and the following seven column headings: Type, Layer, AA position, Reference, Variant, Style, and Description</li> </ul>
<p><b>Files</b> – Can .scf files be analyzed in SeqScape software?</p>	<p>No. SeqScape software analyzes only sequencing data in .ab1 files or text sequences.</p>
<p><b>Files</b> – How can I use sample files generated on a Macintosh® computer with SeqScape software?</p>	<p>To use data files generated on a Macintosh computer with SeqScape software, you must convert the files using the SCU (Sample Conversion Utility). This utility is available as a Macintosh application file on the SeqScape Analysis software CD. The SCU must be loaded onto and launched from a Macintosh computer. For more information, see the Read Me file associated with the SCU.</p>
<p><b>Chemistry</b> – What Applied Biosystems chemistries are supported?</p>	<ul style="list-style-type: none"> <li>• BigDye® Terminator v3.1, v3, v2, v1.1, and v1 chemistries</li> <li>• BigDye® Primers and dRhodamine dyes</li> </ul>

Table 4 General questions and answers (*continued*)

Question	Answer
<b>Computer</b> – What are the computer requirements for SeqScape software?	<ul style="list-style-type: none"> <li>• CPU – 733 MHz or faster, single processor</li> <li>• Memory – 512 MB RAM</li> <li>• OS – Windows® 2000 with Service Pack 3, Windows® XP with Service Pack 2 or Windows Vista™</li> <li>• 5-GB hard drive</li> <li>• Pentium® III or IV chip, <i>not</i> Xenon</li> </ul>
<b>Computer</b> – What kind of performance can I expect from my SeqScape software?	<p>That depends on your computer specifications. For example, if your computer has an 850 MHz processor, 256 MB RAM, and a Pentium® III chip and runs on Windows 2000 OS, and you analyze 100 samples files, 10 specimens, and a 1-Kb reference, the analysis time is 2 min.</p> <p><b>Note:</b> If SeqScape software runs slowly, you can improve performance by archiving older projects. To archive projects, select <b>Tools &gt; SeqScape Manager &gt; Project &gt; Export</b>.</p>
<b>Software</b> – Does SeqScape software support BioLIMS/Sequence Collector software?	No. SeqScape software no longer supports BioLIMS/Sequence Collector software.
<b>Software</b> – How does SeqScape software compare to MicroSeq® and ViroSeq® software?	<p>SeqScape software – Compares samples to a reference sequence</p> <p>MicroSeq software – Identifies bacteria</p> <p>ViroSeq software – Identifies genotype HIV-1 resistance mutations</p>
<b>Software</b> – Do I need Sequencing Analysis software if I have SeqScape software?	<p>Sequencing Analysis software is a multi-purpose software used to analyze, edit, view, display, and print sequencing sample files. Use Sequencing Analysis software for general troubleshooting and viewing of data.</p> <p>SeqScape software is designed specifically for resequencing.</p>
<b>Data objects</b> – Can I transfer data objects like the RDG, Display Settings, Analysis Protocols, etc. from one computer to another?	Yes. You can transfer data objects from one computer to another. To export the data object to the SeqScape Manager, select the object, then click <b>Export</b> . Send the exported file to the second computer, then launch SeqScape software. Open SeqScape Manager, then click <b>Import</b> to import the file.
Can I BLAST against a database?	Yes. 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 <b>File &gt; Export</b> . Open the file in a text viewer, then cut and paste the sequence you want to search for in your BLAST query. Refer to the <i>SeqScape Software User Guide</i> , Chapter 9, “Electronic Signing, Exporting, and Printing Data and Reports,” for details on exporting.

Table 4 General questions and answers (*continued*)

Question	Answer
<b>Alignment</b> – What alignment algorithms are used in SeqScape software?	The sample assembly and specimen alignments are generated using a Smith-Waterman local sequence alignment algorithm with parameters appropriate for DNA sequencing.
<b>Alignment</b> – Can SeqScape software perform just the alignment for samples without basecalling?	Yes. To assemble and analyze sequences without basecalling, open the Analysis Defaults for the project, then select the <b>Specimen</b> tab, then deselect <b>Basecall Samples</b> .

<sup>a.</sup>The imported sequence shows a summary of all the sequences in the file by substituting the IUPAC codes for bases where there is a discrepancy in the sequences.

## SeqScape Manager FAQs

Table 5 SeqScape Manager questions and answers

Question	Answer
What is the SeqScape Manager?	SeqScape Manager is a tool for importing, exporting, creating, and deleting projects, project templates, reference data groups, analysis defaults, libraries, analysis protocols, and display settings.  To access SeqScape Manager, open SeqScape software and select <b>Tools &gt; SeqScape Manager</b> .
What is an object?	An object is a named collection of data elements used to perform certain functions, for example, analysis protocol.
How do I create a new user?	Log in as an Admin user, then: <ol style="list-style-type: none"> <li>1. Select <b>Tools &gt; Options</b>.</li> <li>2. Select the <b>Users</b> tab, then click <b>New</b>.</li> <li>3. Enter the new user name (be sure to omit any spaces in the user name), then click <b>OK</b>.</li> <li>4. Exit the software, then relaunch it.</li> <li>5. Log in with the new user name.</li> </ol>
What is a project in SeqScape software?	Projects contain sample data files grouped into specimens. A project is created using a project template.

Table 5 SeqScape Manager questions and answers (*continued*)

Question	Answer
What is a project template?	A project template is the mold from which projects are created. A project template contains analysis defaults, display settings, and a reference data group.
What is a specimen?	<p>A specimen contains all the sample data from a single biological source.</p> <p>SeqScape software assembles all sample data within a specimen and generates a consensus sequence. For example, a specimen contains forward-strand PCR products for exons 3, 4 and 5 of a gene and several reverse-strand PCR products for the same exons. The software generates a single consensus sequence representing exons 3,4 and 5 and compares it to the reference sequence.</p> <p><b>IMPORTANT!</b> Do not mix products from different biological sources into a single specimen.</p>
What is a layer?	<p>A layer is a set of regions of interest (ROIs) that are grouped together for the purpose of display, report, or amino acid translation. A layer can represent a transcript. The ROIs within a layer cannot overlap.</p> <p>Example: Your project may contain introns 1, 2, 3, 4, 5, 6 and exons 1, 2, 3, 4, 5. You can create a layer that contains only exons 1, 4, 5 or a layer containing intron1, exon1, intron2, exon2, intron3, exon3, and intron4.</p>
How do I generate a new layer?	To generate a new layer, click <b>New Layer</b> in the ROI tab of the RDG, then add the desired ROIs by selecting the <b>On Layer</b> check box in the ROI table. Also, select whether or not you want the ROI translation turned on in the layer.
Can I put samples from different individuals in the same specimen?	No. Each individual sample should be in a different specimen. Refer to the <i>SeqScape Software User Guide</i> .
Can I mix samples from different biological sources?	No. You cannot analyze data from different biological sources in the same specimen.
What is a reference data group (RDG)?	An RDG is an essential part of the project template. An RDG contains all 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.

Table 5 SeqScape Manager questions and answers (continued)

Question	Answer
What are the new features of the extended RDG?	When using a Genbank file to create the reference sequence, the feature table of the Genbank file is pulled into the RDG, and each feature is listed out in the ROI (region of interest) table. The ROIs can be used to create new layers for sequence comparison. For example, if a Genbank file for a gene containing two exons and one intron is imported into the RDG, you can create a layer that includes only the two exons. When analysis occurs, the specimens are compared to the layer containing the two exons as well as to the reference backbone layer that includes the two exons and the intron. In addition, you can turn translation on or off for specific ROIs. A library containing aligned sequences can also be attached to a specific layer for comparison during analysis.
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 section of the reference backbone within the reference sequence that corresponds to a single contiguous DNA sequence.
What is the reference backbone?	The reference backbone is the entire reference sequence that can consist of one or more reference segments. The backbone is the first layer of the RDG, which cannot be modified.
What does splitting the reference segment mean and how do I split it?	A reference segment is split when the sequences are not contiguous; For example, one side of the split may contain Exon3 and the other side may represent Exon8. To split a reference segment, in the ROI tab, click the base position where you want to split the reference segment, then select <b>Split Reference Segment</b> .
Where can I find information on the ROI tab?	You can find information by clicking <b>Info</b> on the bottom left of the ROI tab within the RDG.
Can the ROI contain negative numbering?	Yes. You can assign a negative number to an ROI by entering the number into the ROI table of the RDG.
Can the reference sequence contain negative numbering?	No. The reference backbone sequence cannot contain negative numbering. However, individual ROIs within the reference backbone can contain negative numbers.

Table 5 SeqScape Manager questions and answers (*continued*)

Question	Answer
How do I save GenBank files?	After finding the desired sequence at the NCBI website, select the check box to the left of the accession number. At the top of the page next to Display, select <b>GenBank</b> , then select <b>Send to File</b> . The file is saved to the specified location and can then be imported into the RDG. The file can have a .gb, .fcgi, or .cgi extension.
How can I designate part of my sequence as untranslated (intronic region)?	First, select the desired section of the sequence in the ROI tab, then click <b>Add ROI</b> . The region appears in the ROI table. Select the layer where you want the ROI to appear, then deselect the <b>Translate</b> check box.
How can I change the number of the first base in the reference sequence? How can I reset the first codon?	You can designate the first base/codon in the reference segment pane of the ROI tab. This is the pane that shows selected reference sequence. Click the box on the top left of the pane, then enter the desired number.
How can I change the translation frame?	You can change the translation frame in the ROI tab of the RDG.
Can I use an implicit reference sequence in SeqScape software?	No. SeqScape software does not support the use of an implicit reference sequence. However, you can use .ab1 files and genbank files as reference sequences.
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 the <i>SeqScape Software User Guide</i> , Appendix E, "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. The numbering for the ROI is continuous, always increasing from left to right. ROI does not have to correspond to the numbering on the reference segment. The ROI can also contain negative numbers. ROIs can be grouped into layers for display or translation purposes

Table 5 SeqScape Manager questions and answers (continued)

Question	Answer
How can I configure a reference segment and the 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.
<b>File import</b> – What kinds of files can I import into SeqScape software?	AB sample files, tab-delimited text, and FASTA file format can be imported into the software.
<b>File import</b> – Can I import multiple individual text files into one specimen rather than .ab1 files?	Yes. You can import .seq format files or FASTA format files as sample files. To see these files in the import dialog box, deselect <b>Show .ab1 Samples File Only</b> . The files can then be analyzed (no basecalling occurs).
<b>File import</b> – Do my sample files need to be imported in the same reading frame as the reference sequence?	No.
<b>File format</b> – Can analyzed data be used in SeqScape software?	Yes. Analyzed data can be used. However, if the data are in the ABI data format (not FASTA), any prior analysis, results, and edits are overwritten when the files are reanalyzed using SeqScape software.
<b>File format</b> – 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.  <b>Note:</b> 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)  aatactcaaatgggcctgtcctttagtataaactaatacacagcttctgtaaacgggatg aaaacctttccaaggacaatcagagaaaaagtctttaactccaccattagcaccxaaag ct (hard return)
What are Analysis Settings?	The analysis settings specify the basecalling, mixed base settings, clear range, and filter settings.
What is Clear Range?	The range of usable sample sequence to be included in the consensus.

Table 5 SeqScape Manager questions and answers (*continued*)

Question	Answer
<p>Can the Clear Range be modified within a project? Does changing the Clear Range require that the data be reanalyzed?</p>	<p>You can change the Clear Range for the entire project by applying a new Project Template (with a modified Analysis Protocol), in which case all samples are reanalyzed and any sample basecall/edits are lost.</p> <p>You can reset the Clear Range for an individual sample by modifying the sample's Analysis Protocol setting. In this case, the specimen containing affected sample(s) must be re-analyzed. If only the Clear Range tab was modified in the Analysis Protocol, the analysis pipeline is started from the Clear Range determination onward, so basecalls are not overwritten.</p> <p>You can also change the sample Clear Range within the sample file without reanalyzing: Right-click a sample sequence and select <b>Set CR... at selection</b>, then click-drag the CR bracket to reset the Clear Range. You can also select <b>Tools &gt; Set Clear Range</b> to reset the sample clear range.</p>
<p>What are Filter Settings?</p>	<p>Filter settings specify the maximum percentage of mixed-bases allowed, maximum Ns allowed, minimum clear range length, and the minimum sample score for each sample.</p> <p>Samples failing the filter checks are not included in the analysis.</p>
<p>What are Display Settings?</p>	<p>Display settings control the font styles and colors for bases, electropherogram display and axis scale, display view for variants, and display views for nucleotide translation.</p>
<p>Can I export consensus sequences?</p>	<p>Yes. Select the desired consensus sequence in the Project Navigator, then select <b>File &gt; Export</b>.</p>

## Library FAQs

Table 6 Library questions and answers

Question	Answer
<p>What are the requirements of the library?</p>	<p>The library search feature is designed for use with libraries of alleles, genotypes, or haplotypes where all the sequences in the library are variations of the same sequence. This feature is not designed for searching against a library of diverse sequences. The library must have the following characteristics:</p> <ul style="list-style-type: none"> <li>• All library sequences must be pre-aligned and equal in length.</li> <li>• All library sequences must be variations of the same sequence (variations must be less than 50%).</li> <li>• All library sequences must cover the same regions as the layer that is associated with that library (for example, for a layer that contains exons 2, 3, 7 and 8, a valid library should have sequences from exons 2, 3, 7, and 8. A library with sequences covering exons 2,3, 4, 5, 7, and 8 would be invalid).</li> </ul> <p>A library is associated with its specific layer.</p>
<p>Is there a minimum/maximum number of Library Search match returns that I can define?</p>	<p>No. There is no maximum. However, it does not make sense to request more than the number of alleles in the library. The minimum should be 1. You can set the number of library matches to return in the Analysis Defaults &gt; Specimen tab of the project.</p>
<p>What is the difference between a haploid and diploid library?</p>	<p>In a haploid library, all the sequences are pure base sequences. A diploid library contains both mixed base and pure base sequences. A haploid library returns two possible matches, while a diploid library returns one possible match.</p>
<p>Can I add a library to an open project from within the RDG properties button and see the search results instantly, or must I re-analyze the project for the library search to be initiated?</p>	<p>The library is automatically searched immediately after loading a new library for the active layer and after closing the RDG dialog box.</p>
<p>What is the function of the Library Identification pane? How can I view the Library Identification pane?</p>	<p>You can use the Library Identification pane to display the crucial positions identified among the set of library matches returned against the selected specimen consensus sequence. To view this pane, click a base in a specimen consensus sequence in the Project Assembly view. You can adjust the height of the pane using the drag-split bar. The crucial position columns are hyperlinked to the specimen consensus sequence base positions that are highlighted by the column selector in the Project Assembly view.</p>

Table 6 Library questions and answers (continued)

Question	Answer
What is a crucial position error?	A crucial position error is a polymorphic position that occurs in all the allelic matches. It is the position that makes each allele unique.
What is a constant position error?	A constant position error is a position in a specimen consensus sequence that is different from the corresponding position in all the allelic matches. All the allelic matches have the same base for that particular position.

## Mutation, Variant, HIM, and HFM Detection FAQs

Table 7 Mutation, Variant, HIM, and HFM Detection questions and answers

Question	Answer
Does SeqScape software account for heterozygous indel mutations (HIMs) (for example, a sequence with an insertion of three bases)?	Yes. SeqScape software shows possible HIM location and identity in the Analysis QC Report.
Can the SeqScape software separate the HIM sequence traces?	No. SeqScape software cannot separate HIM sequence traces. However, SeqScape software reports how many bases were deleted or inserted.

## Data Analysis FAQs

Table 8 Data Analysis questions and answers


Question	Answer
How do I begin analysis?	Click  (Analyze) in the toolbar or select <b>Analysis &gt; Analyze</b> .
Can the SeqScape software handle gaps in sequence?	SeqScape software automatically inserts gaps in the sample and consensus sequences if the gaps are necessary to produce clean sequence alignments. Remove gaps 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?	Edited bases are displayed as lowercase letters; unedited bases are displayed in uppercase letters.
What happens to my edited sequence when I start analysis?	After basecalling starts, all current edits are overwritten. 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.
How do I remove unwanted spaces in my samples?	To remove unwanted spaces in the sample, double-click the space, then press the <b>Delete</b> key.
What can I do if I deleted too many bases?	Repeat the analysis.
Is there an option to basecall without generating quality values?	No. All the basecallers in SeqScape software generate quality values. However, you do not have to display the quality values. You can hide the quality values by deselecting the confidence bar icons in the Views tab of the project's Display Settings.

Table 8 Data Analysis questions and answers (continued)

Question	Answer
Can I assemble/analyze my samples without re-basecalling my samples so that I can conserve the existing basecalls?	Yes. To assemble and analyze your sequences without basecalling, open the Analysis Defaults for the project, select the <b>Specimen</b> tab, then deselect <b>Basecall Samples</b> .
How do I analyze samples in one project with different basecallers/dye set primer files?	Select <b>Analysis &gt; Sample Manager</b> , select the appropriate basecaller and dye set/primer files, then click <b>Apply</b> . The project must be re-analyzed for the changes to take effect. Samples and specimens with a red slash indicate an unanalyzed status.  In the Sample Manager, you can also edit the analysis protocol for the individual samples or apply an analysis protocol.
What does a red line through the specimen icon indicate?	A red line indicates that analysis has not occurred. A red line may also appear if the analysis settings have been changed and the project requires re-analysis to apply the settings. Click the green arrow (run) button at the top of the window to start analysis.
How can I edit my specimen name?	Select the specimen, then select <b>Edit &gt; Rename</b> or right-click the selected specimen, then select <b>Edit</b> .
How can I delete samples or specimens?	Select the item to be deleted, then do one of the following: <ul style="list-style-type: none"> <li>• Select <b>Edit &gt; Delete</b>, click the <b>Delete</b> button on the toolbar, press the <b>Delete</b> key on the keyboard.</li> <li>• Or right-click the selected item, then click <b>Delete</b>.</li> </ul>
What is the TraceTuner™ basecaller module?	The ABI basecaller contains an algorithm that assigns bases and invokes a second algorithm, the TraceTuner module. The TraceTuner module generates per-base sample quality values and identifies mixed bases.
What does a red dot mean in the analyzed project?	A red dot indicates a base that has been called by the consensus caller. The consensus caller edits this base in the relevant sample sequences of the specimen. The edited base appears in lowercase, and has a gray quality-value bar.
Can I change the settings of the tab jump key?	You can change the settings of the tab jump key in the Views tab of the project Display Settings. When the project is open, you can also change the tab jump key settings by selecting the Tab jumps to next... option on the toolbar.

## Analysis Reports FAQs

Table 9 Analysis reports questions and answers

Question	Answer
How can I access my reports?	Access all reports by clicking the <b>Report Manager</b> button in the toolbar or selecting <b>Analysis &gt; Report Manager</b> .
What are the different reports available in SeqScape software?	SeqScape software v2.6 can generate the following reports: <ul style="list-style-type: none"> <li>• Analysis QC Report</li> <li>• Mutations Report</li> <li>• AA Variants Report</li> <li>• Specimen Statistics Report</li> <li>• Sequence Confirmation Report</li> <li>• Base Frequency Report</li> <li>• Library Search Report</li> <li>• RDG Report</li> <li>• Audit Trail Report</li> <li>• Electronic Signature History Report</li> <li>• Genotyping Report</li> </ul>
What is the Analysis QC Report?	The Analysis QC report provides a summary of the project's history. This report indicates the status of each specimen at each step of analysis. In addition, the Analysis QC report lists possible HIMs (heterozygous insertion/deletion mutations).
What does “Segment Score” mean in the Specimen Statistics report?	Segment Score gives an average of all the quality values within the clear range in that particular reference segment region.
What does “Coverage” mean in the Specimen Statistics and Sequence Confirmation reports?	Coverage gives a value for the number of samples in the consensus sequence.
Can I edit sequences within a project while reports are open and see the updated information in the reports instantly, or must I close and re-open the reports to see any changes?	You can see updated information as you edit.

Table 9 Analysis reports questions and answers (continued)

Question	Answer
Why are my sample files unassembled?	If you have samples in the unassembled node of a specimen, check the Analysis QC report to determine why the sample files were not assembled. The analysis QC report shows whether or not the sample assembled, as well as the reason for failure at a particular point in the analysis pipeline.

## Quality Values FAQs

Table 10 Quality value questions and answers

Question	Answer
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).
Is there an option to basecall without generating quality values?	No. All the basecallers in SeqScape software generate quality values. However, you can choose not to display the quality values by deselecting the confidence bar icons in the Views tab of the project's Display Settings.
How is the basecaller quality value generated?	The basecaller quality value is generated by an algorithm that is designed to examine the certainty of basecalls. See the <i>SeqScape Software User Guide</i> , Appendix A, "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 by a statistical algorithm that 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.

## Printing and Exporting Results FAQs

Table 11 Printing and exporting results questions and answers

Question	Answer
<b>Printing</b> – What can I print in SeqScape software?	You can print the views only for sample, specimen, segment, project, and complete reports. You can also print electropherograms, complete reports, and the visible data or all data for the project view.
<b>Printing</b> – What printers are recommended for use with SeqScape software?	An HP® 6122, 6940, 4600, 8100, 990cxi, or an Epson® 980 color printer is recommended
<b>File export</b> – 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 the <i>SeqScape Software User Guide</i> , Appendix F, “User Privileges.”
<b>File export</b> – Can I export each consensus sequence individually?	Consensus sequences for a project can be exported as a group by selecting <b>File &gt; Export</b> in the Project view.
What format can I print/export reports in?	You can export reports in .pdf, .xml, .htm, or .txt file formats. You can print the exported reports or you can print an open report by selecting <b>File &gt; Print</b> .
Can I export and print individual .ab1 sample files from the project?	Yes. Select the sample file in the Project Navigator view, then click a sample file and select <b>File &gt; Export &gt; Sample Sequence File</b> . The sample file can be exported in four formats: .seq, .fasta (FASTA), .phd.1 (PHD), and .ab1. You can print individual sample files by selecting <b>File &gt; Print</b> .

# Audit Trail, Security, and Access Control FAQs

Table 12 Audit trail, security, and access control questions and answers

Question	Answer
<p>What security and audit trail features are included in SeqScape software?</p>	<p>SeqScape software v2.6 has the following security and audit trail features:</p> <ul style="list-style-type: none"> <li>• Three levels of user access</li> <li>• User lockout after a specified time frame has passed</li> <li>• Password expiration</li> <li>• Audit trail that can be created for base change, insertion, or deletion</li> <li>• Audit trail that includes time/date stamp and reason for change</li> <li>• User name that is displayed when logged into the software</li> <li>• Audit Trail report</li> </ul>
<p>What are the access control differences as you go from Admin to Scientist to Analyst?</p>	<p><b>Administrators</b> can do everything that the application possibly lets you do, including the admin-specific tasks: Creating users, viewing and changing user details, importing and exporting users, and changing the Authentication and Audit features that assist with 21 CFR Part 11 requirements.</p> <p><b>Scientists</b> can do everything except the admin-specific tasks.</p> <p><b>Analysts</b> can open projects and import samples, but cannot affect other master objects. For example, analysts cannot view, modify, import, or export project templates, RDGs, or analysis defaults. Analysts cannot change the RDG or analysis settings in a project, although they can change the basecaller and dye set/primer files from within the Sample Manager. Analysts also cannot perform any admin-specific tasks. Analysts are allowed to edit the project.</p> <p>For more information, see the <i>SeqScape Software User Guide</i>, Appendix F, “User Privileges.”</p>
<p>Does the audit trail function add User ID and a Time/Date stamp to each entry?</p>	<p>Yes. The Audit Trail report does include a user ID, user first and last names, and time/date stamp for each audit event. The Audit Trail report also includes the reason why the user modified the data, and it includes any comments entered.</p>

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