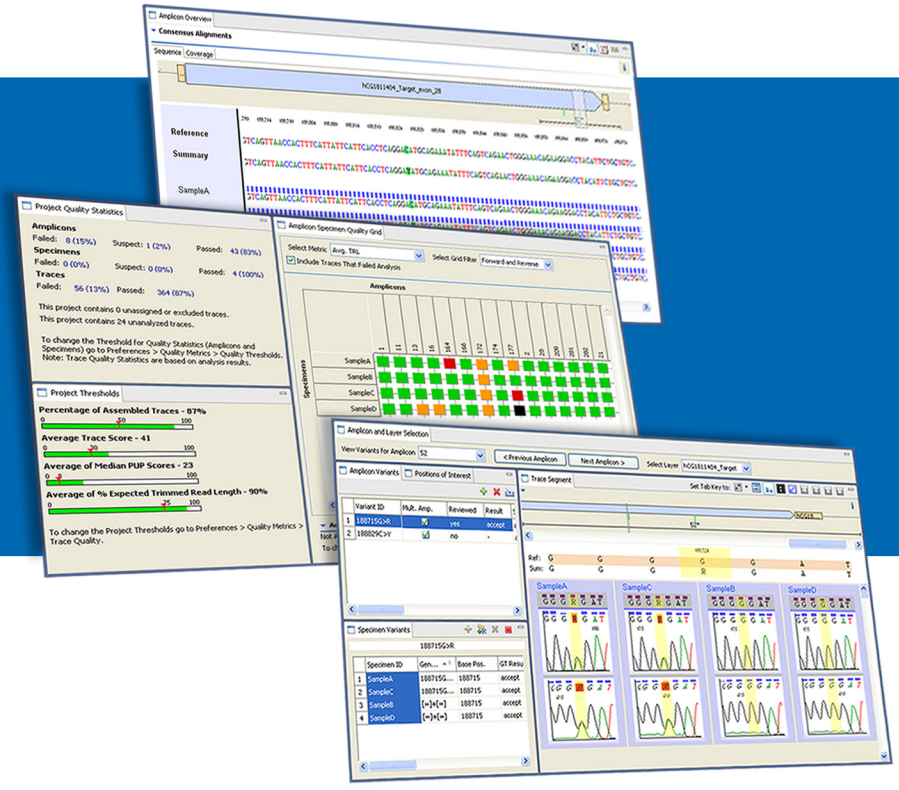
Applied Biosystems

Applied Biosystems Variant Reporter™ Software

Version 1.1



Applied Biosystems

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Set Up a Project 3

Review a Project 4

Variant Reporter™ Software

Version 1.1

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Preface

How to use this guide

Purpose of this guide

The *Applied Biosystems Variant Reporter™ Software v1.1 Getting Started Guide* is an installation guide and tutorial. It provides step-by-step instructions for installing Variant Reporter™ Software, setting up a project based on trace data, and analyzing that project. It is designed to help you quickly learn how to use the Variant Reporter™ software.

Audience

This guide is intended for new users of the Variant Reporter™ Software, including research scientists and sequencing analysts.

Assumptions

This guide uses conventions and terminology that assume a working knowledge of the Microsoft® Windows® operating system and/or the Microsoft® Windows® XP operating system.

Text conventions

This guide uses the following conventions:

- **Bold** text indicates user action. For example:

  Type **0**, then press **Enter** for each of the remaining fields.
- *Italic* text indicates new or important words and is also used for emphasis. For example:

  Before analyzing, *always* pre-basecall sequence data.
- A right arrow symbol (▸) separates successive commands you select from a drop-down or shortcut menu. For example:

  Select **File ▸ Open ▸ Spot Set**.

  Right-click the sample row, then select **View Filter ▸ View All Runs**.

User attention words

Two user attention words appear in Applied Biosystems user documentation. Each word implies a particular level of observation or action as described below:

Note: – Provides information that may be of interest or help but is not critical to the use of the product.

IMPORTANT! – Provides information that is necessary for proper instrument operation, accurate chemistry kit use, or safe use of a chemical.

Examples of the user attention words appear below:

Note: The reference can be copied to the Dashboard for future use.

IMPORTANT! Always import pre-basecalled trace data for optimum analysis results.

How to obtain more information

Related documentation

The following related documents are shipped with the system:

- ***Variant Reporter™ Software v1.1 Help*** **–** Describes the Variant Reporter™ software and provides procedures for a recommended workflow. The Help is accessed in the software menu ( ).
- ***Variant Reporter™ Software v1.1 Getting Started Guide*** **–** Provides instructions for installing the software and setting up and analyzing a project in the Variant Reporter™ software.
- ***Variant Reporter™ Software v1.1 Quick Reference Cards*** **–** Provide an overview of the two types of Variant Reporter™ workflows and briefly takes the analyst through each workflow.

Portable document format (PDF) versions of this guide and the *Variant Reporter™ Software v1.1 Quick Reference Cards* are also available on the software CD.

Note: To open the user documentation included on the Documentation CD, use the Adobe® Acrobat® Reader® software available from **www.adobe.com**.

Note: For additional documentation, see "How to obtain support" on page x.

Obtaining information from the help system

The Variant Reporter™ Software v1.1 features an online Help system that describes how to use each feature of the user interface. Access online Help by opening the software and doing one of the following:

- Click in the toolbar of the Variant Reporter™ Software
- Select **How Do I?**

You can use the Help system to find topics of interest by:

- Reviewing the table of contents
- Searching for a specific topic

You can also access PDF versions of all documents in the Variant Reporter™ Software Version 1.1 document set from **Start** ▸ **Programs** ▸ **Applied Biosystems** ▸ **Variant Reporter** and then select the appropriate PDF document.

Send us your comments

Applied Biosystems welcomes your comments and suggestions for improving its user documents. You can e-mail your comments to:

techpubs@appliedbiosystems.com

IMPORTANT! The e-mail address above is only for submitting comments and suggestions relating to documentation. To order documents, download PDF files, or for help with a technical question, go to **www.appliedbiosystems.com**, then click the link for **Support**. (See "How to obtain support" below).

How to obtain support

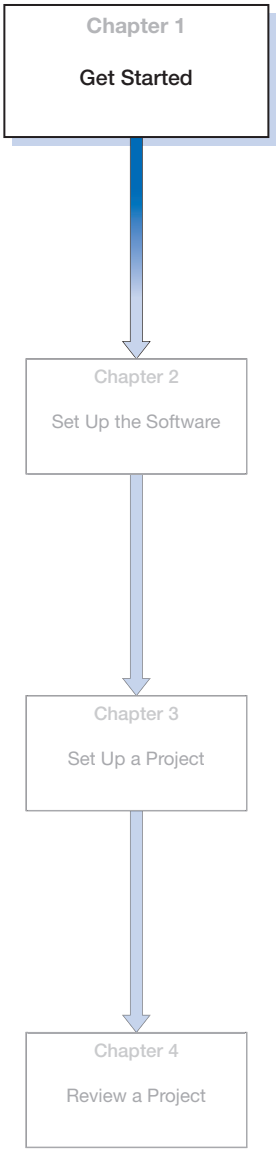
For the latest services and support information for all locations, go to **www.appliedbiosystems.com**, then click the link for **Support**.

At the Support page, you can:

- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support
- Order Applied Biosystems user documents, MSDSs, certificates of analysis, and other related documents
- Download PDF documents
- Obtain information about customer training
- Download software updates and patches

In addition, the Support page provides access to worldwide telephone and fax numbers to contact Applied Biosystems Technical Support and Sales facilities.

1 Get Started



This chapter covers:

- About Variant Reporter™ software 2
- About task-driven workflow 3
- About tutorial data 4
- Software user interface overview 5

About Variant Reporter™ Software

Overview

Applied Biosystems Variant Reporter™ Software v1.1 is a variant detection and reporting software for resequencing applications such as mutation detection and analysis and SNP discovery and validation. The Variant Reporter™ software enables researchers and clinicians to view, edit, print, and export sequence data generated by Applied Biosystems genetic analyzer instruments. This software effectively reduces the workflow bottleneck caused by researchers' time-intensive data analysis and review cycles.

Direct sequencing has created the need for more accurate variant detection in research and clinical diagnostics. Increasing confidence can come from applying strict quality control metrics, including the use of quality values for DNA trace values and confidence scores for variant validity. By applying specific analysis parameters for trimming and filtering, the Variant Reporter™ software removes low quality data, allowing reviewers to focus only on those variants with low confidence scores.

Features of Variant Reporter™ software

Version 1.1 of the Applied Biosystems Variant Reporter™ software has the following features:

- Dashboard View for instant viewing of all projects
- Project View with streamlined, task-focused, intuitive workflow
- Targeted variant presentation to optimize user review time
- Flexibility to analyze traces with or without a reference
- In-depth, quality summaries from the project level to trace level
- 3 project reports; 7 quality reports – All reporting has comprehensive export capability
- New algorithms that ensure high confidence results
- Drag-and-drop functionality – Take data and move it easily between windows
- Frequently Asked Questions and comprehensive Help

About task-driven workflow

Project workflow

The project workflow shown below provides an overview of the main tasks that you perform using this *Getting Started Guide*. This workflow represents a typical workflow you perform when working in the Variant Reporter™ software.

Note: The numbered steps in the flowchart are mandatory and the unnumbered steps are optional.

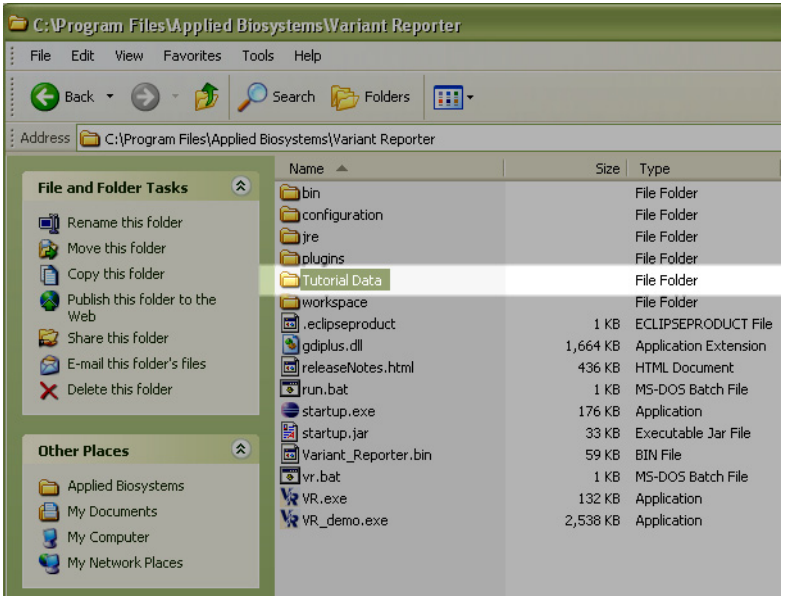
About tutorial data

Locate tutorial data

When you perform the tasks in this *Getting Started Guide*, you will use tutorial data that is supplied on the Variant Reporter™ software CD. The tutorial data installs on the following drive location:

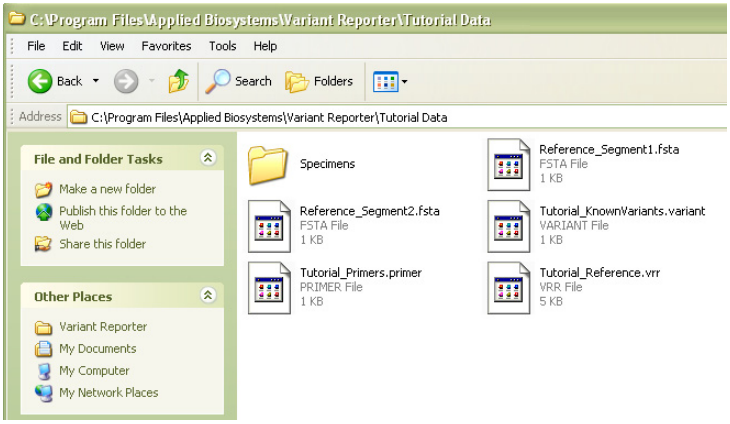
D:\AppliedBiosystems\Variant Reporter\Tutorial Data

Note: If you install Variant Reporter™ Software v1.1 on a drive other than D, go to that drive to find the tutorial files.



The contents of the **Tutorial Data** folder are:

- Specimens (24 traces with 6 specimens and 2 amplicons)
- Reference segments (2 .fasta files)
- Primer file
- Known variant file (with a substitution, deletion, and insertion)
- Complete .vrr file (Variant Reporter™ software reference file)



Find the tutorial data in the **Specimens** folder. The folder contains all the files that you will use to get started.

In Chapter 3, "Set Up a Project," you will follow the recommended workflow, from importing traces to analyzing sample data.
In Chapter 4, "Review a Project," you will review the quality results, then narrow your focus to the variant review phase.

Software user interface overview

Overview of Variant Reporter™ software views

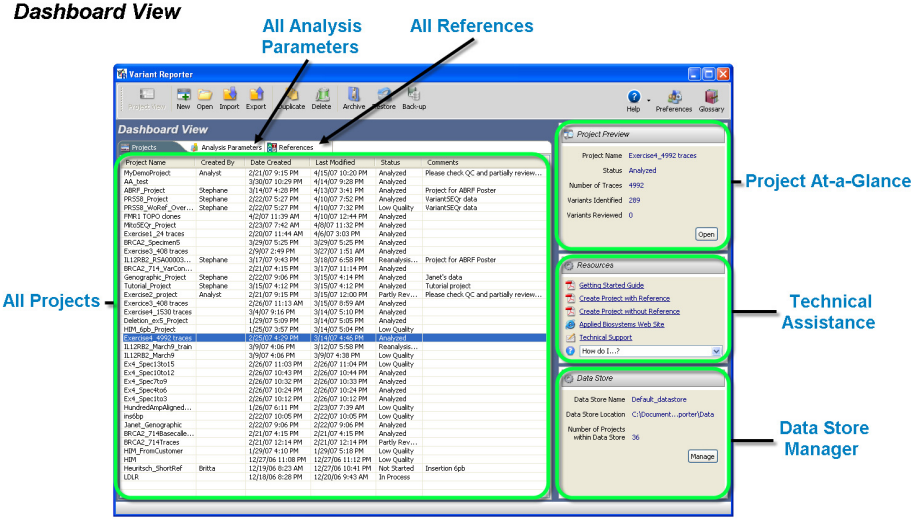
There are two main views in Variant Reporter™ software – the **Dashboard View** and the **Project View**. The Dashboard houses a complete list of all projects, references and analysis parameters that have been created and saved to date. The Project View page is the work space where you create projects and direct tasks.

Dashboard View

From the Dashboard View you can:

- Create a new project
- Import a project, reference or analysis parameter
- Find a previously saved project, reference or analysis parameter
- See a preview of any selected project
- Get technical resources, such as links to documentation
- View and back-up your Data Store location

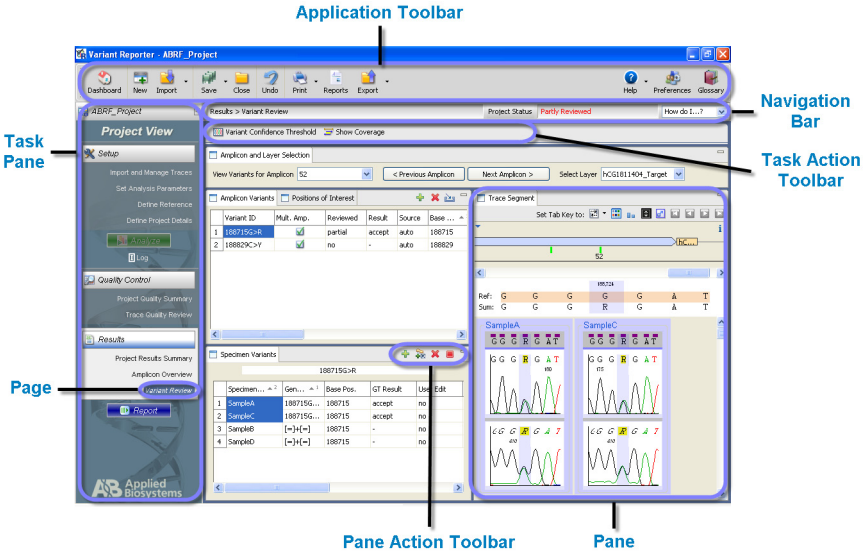
Dashboard View



Project View

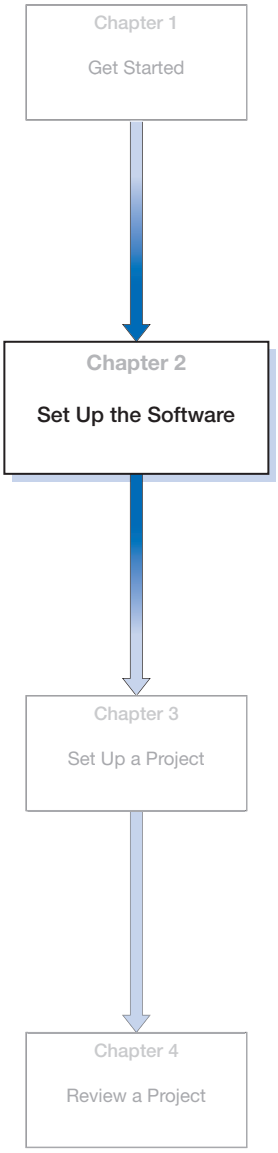
From the Project View you can:

- Import and manage traces
- Set analysis parameters
- Define a reference for the project
- Specify project details
- Analyze
- View project quality and results
- View amplicons
- View variants
- Print and export reports



Refer to "Optimize Variant Reporter™ performance" on page 11 to learn the steps you can take to increase the efficiency of the software.

2 Set Up the Software



This chapter covers:

- Before you begin. 10
- System specifications and performance 11
- Tips for optimizing performance 11
- Install and start Variant Reporter™ Software v1.1. 13

Before you begin

System specifications and performance

This section describes the minimum hardware, software and network requirements for running the Variant Reporter™ Software v1.1. It also contains recommendations for additional peripheral devices for data storage, electrical protection, and network security.

Minimum requirements

The following table lists the minimum requirements for running version 1.1 of the software.

Component	Minimum Requirements
Computer	• Processor, 2.8 GHz
Monitor	• 1024 x 768 • 17-inch color monitor
Memory	• Minimum requirement: 512MB RAM • Recommended performance: 1 GB RAM
Operating System	• Microsoft Windows® XP Professional Operating System, Service Pack 3 • Microsoft Windows® Vista™ Business Operating System, Service Pack 1

Note: Minimal testing was performed on Windows® Vista™ Professional operating system.

Network requirements

The Variant Reporter™ software operates within the Windows® environment. If you plan to connect the computer running Variant Reporter™ software to a network, complete the installation of Variant Reporter™ software *before* configuring the computer for network use. See "Install Variant Reporter™ software" on page 13.

Tips for optimizing performance

Optimize Variant Reporter™ performance

There are steps you can take to reduce project analysis time before setting up your first project in the software.

To optimize Variant Reporter™ software performance, do this:

- Name sample files in Data Collection using delimiters (./-/_) that are helpful in assigning traces to specimens and amplicons. (Example: SampleID_AmpliconID_orientation.ab1)

  Note: You cannot use foreign characters in trace file names.

- Use Sequencing Analysis v5.4 analysis protocol templates specific to your instrument *or* edit your existing analysis protocol to match the following:
  - Select 'Do not assign Ns to Basecalls'
  - Select 'Use Mixed Base Identification'
- Import *pre-basecalled* sample files into Variant Reporter™ software. See "Pre-basecalled data performance" on page 12.
- Use the Dashboard View to move, copy or delete files from the current Data Store location.

  IMPORTANT! Never copy/paste file directly into the Data Store. Always use the Dashboard to manage the Data Store contents.

Figure 1 Pre-basecalled data performance

Project Size	51 Amplicons	KB v1.4.1 PreBasecalled Traces	Variant Reporter™ Software v1.1 Analysis Time (minutes:seconds)
814 Traces (8 Specimens)	not defined	no	3:17
		yes	0:19
	defined	no	3:00
		yes	**0:19**
1530 Traces (15 Specimens)	not defined	no	7:19
		yes	0:40
	defined	no	7:12
		yes	**0:36**
3162 Traces (28 Specimens)	not defined	no	15:54
		yes	2:47
	defined	no	13:35
		yes	**1:27**
4998 Traces (49 Specimens)	not defined	no	28:35
		yes	7:09
	defined	no	27:56
		yes	**4:40**

# Traces	Basecall Time*
814	6:11
1530	12:28
3162	25:04
4998	42:30

Note: This data performance test was conducted on a system with 2GB of RAM and a 3GHz CPU. The reference contained 14,579bp.

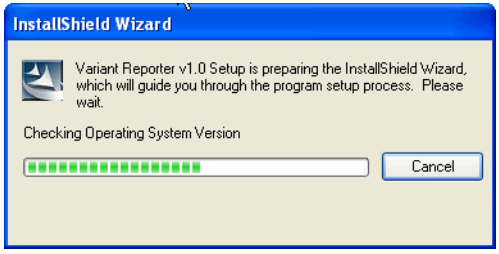
Install and start Variant Reporter™ Software v1.1

IMPORTANT! For optimal performance, install the Variant Reporter™ Software v1.1 on a computer that does *not* run data collection.

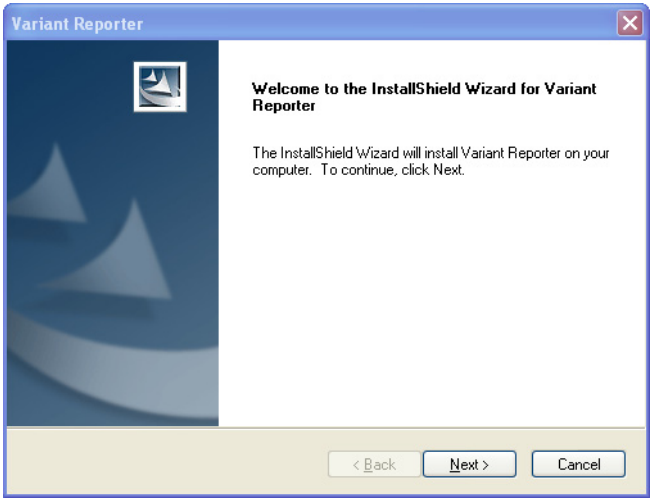
Install Variant Reporter™ software

Follow these instructions to install Variant Reporter™ Software v1.1 on your system.

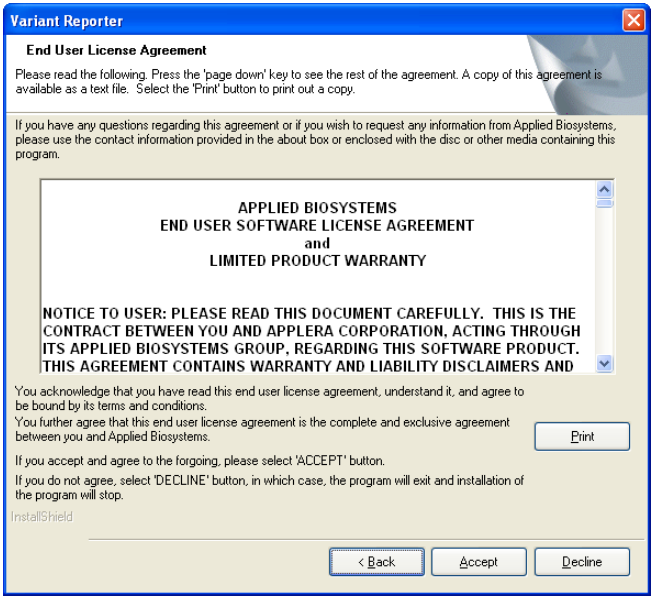
1. Insert the **Variant Reporter™ Software v1.1 CD** to auto-launch the InstallShield Wizard.



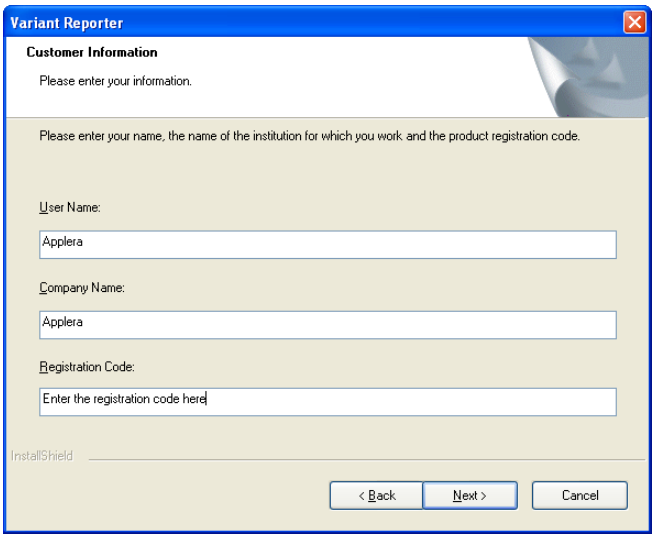
2. At the Welcome screen, click **Next**.



3. In the License Agreement dialog box, scroll to read the license agreement and warranty, then click **Accept**.

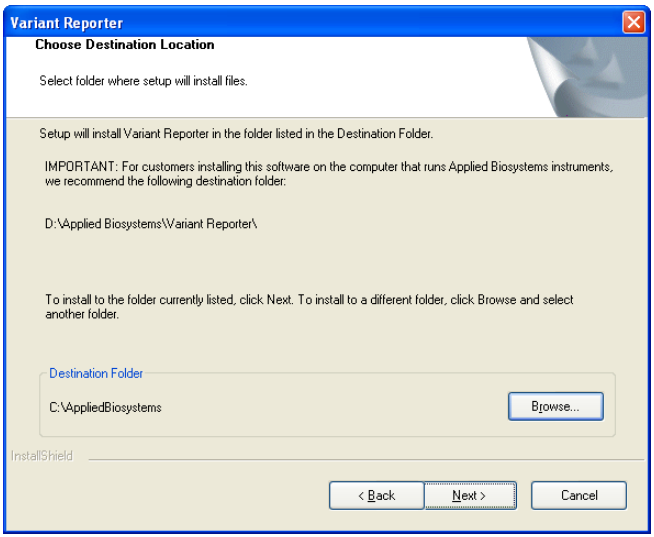


4. In the Customer Information dialog box, enter **User Name**, **Company Name**, and your **Registration Code**, then click **Next**.



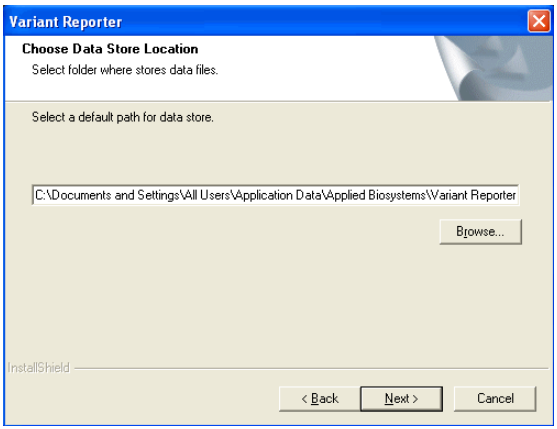
Note: The software registration code is printed on a sticker on the End User Software License Agreement.

5. In the Choose Destination Location, keep the default path, then click **Next.** (Click **Browse** to locate and select a different location for the Variant Reporter™ files to be stored).



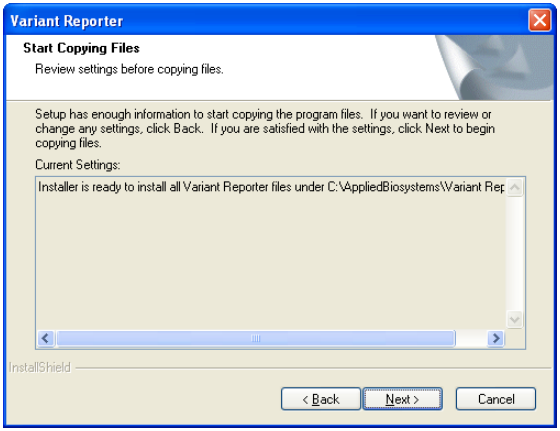
IMPORTANT! If you are installing Variant Reporter™ software on a system running an AB instrument, change the destination folder; install the software on the D drive if using the ABI Prism 310 system, or install on the E drive if using any other AB instrument (3100-Avant, 3100, 3130, 3130xl, 3730, 3730xl system).

6. In the Choose Data Store Location dialog box, select a location on your system where you want the Variant Reporter™ software **Data Store** to reside.



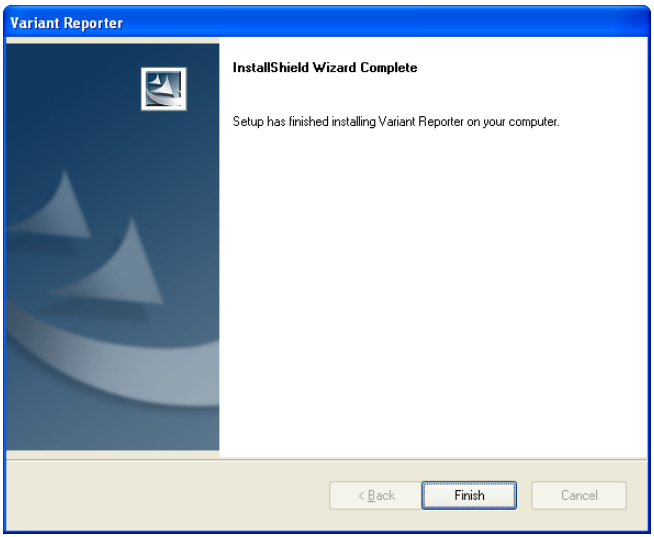
Note: The Data Store location is where all project files are physically stored on your system. The default location is where Windows® programs typically stores your data.

7. In the Start Copying Files dialog box, accept the path you designated for your Data Store location, then click **Next**.



The InstallShield Wizard downloads the files to the selected location.

8. Click **Finish** to complete the installation.



IMPORTANT! The latest version of the KB™ Basecaller (v1.4.1) should already be installed on your Data Collection system or your Sequencing Analysis Software v5.4 for optimal Variant Reporter™ software analysis performance.

Start the software

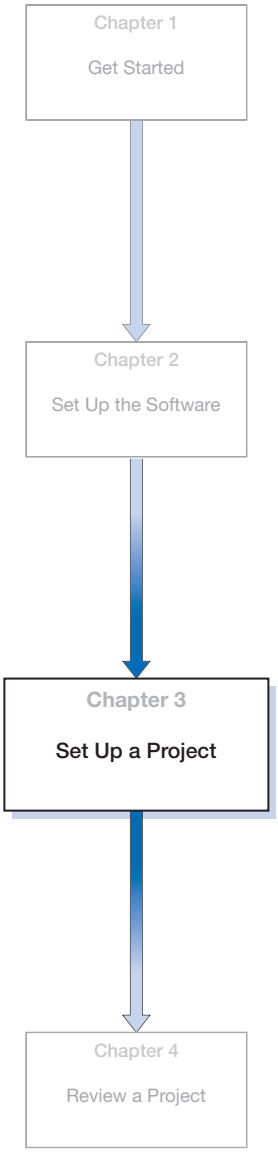
To begin using the software:

Double-click the Variant Reporter™ software desktop icon 

or

Select **Start ▸ Programs ▸ Applied Biosystems ▸ Variant Reporter ▸ Variant Reporter v1.1**.

3 Set Up a Project



This chapter contains:

- Set up a Variant Reporter™ software project 20
- Specify a reference 25
- Set up the amplicons 29
- Create layers and regions of interest (ROIs) 34
- Analyze the project 37

Set up a Variant Reporter™ Software project

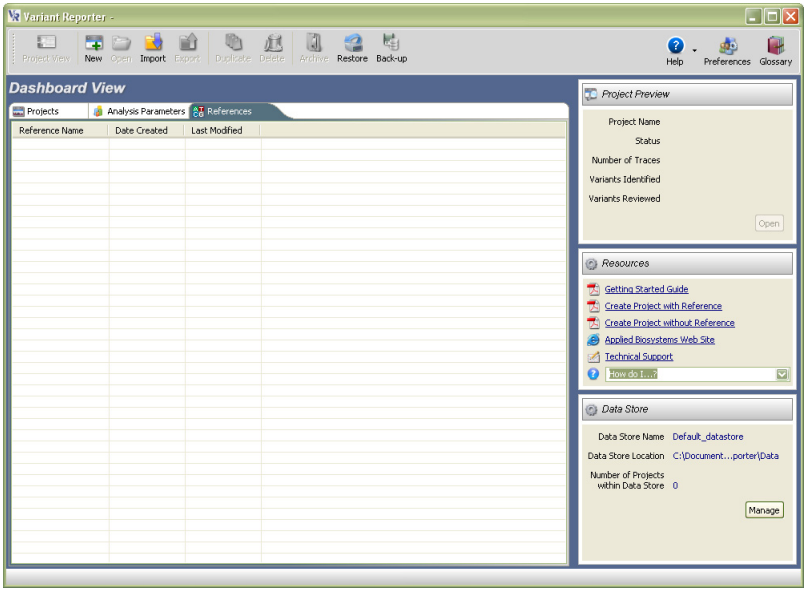
The tasks outlined in this section use tutorial data that is supplied on the software CD.

Import traces into a new project

To begin using the Variant Reporter™ software:

1. Double-click the desktop icon: 

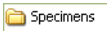
   The software opens to the Dashboard View.

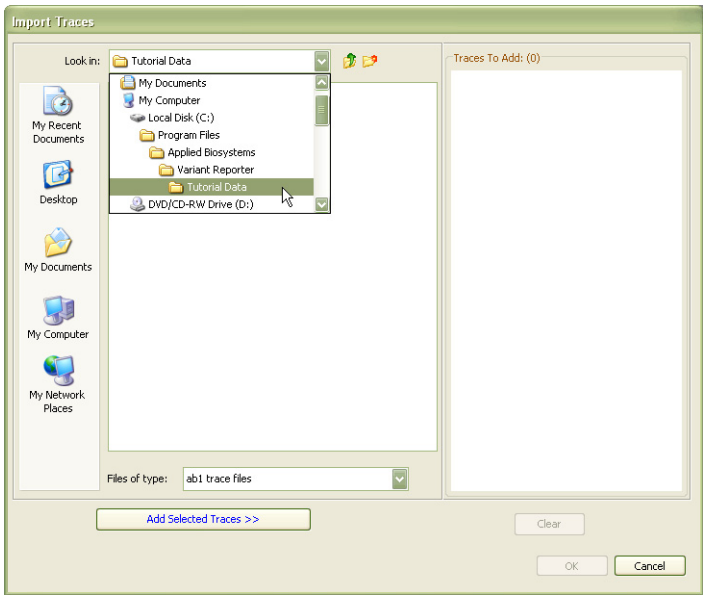


2. Click  on the Application toolbar to begin a new project.

   The Project View opens.

3. Click  (Task Action toolbar) to import traces.

4. In the Import Traces dialog box, navigate to the Tutorial Data folder (**D:\AppliedBiosystems\Variant Reporter\Tutorial Data**), then select the  folder.

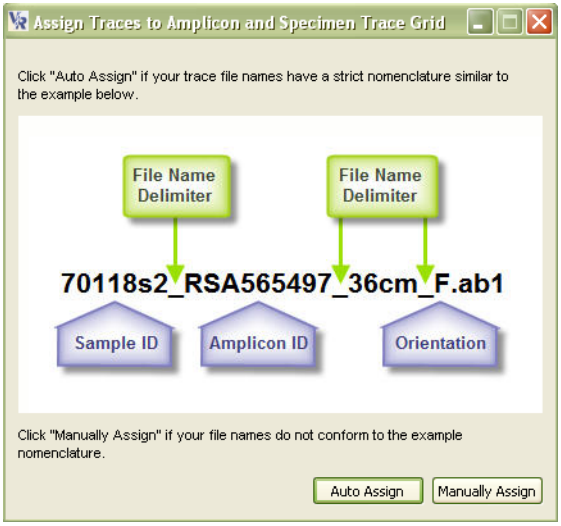


5. Click Add Selected Traces >>, click **Yes** at the prompt, then click **OK** to finish importing the 24 traces.

IMPORTANT! Importing brings a *copy* of the files from your computer into the Variant Reporter™ software. The original files still reside on your system.

Group traces into amplicons and specimens

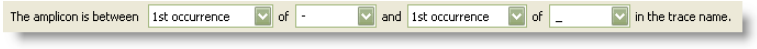
1. In the Assign Traces to Amplicon and Specimen Trace Grid dialog box, determine if your traces have a similar nomenclature to the example shown. If your trace files *are* similar, or if you are using the tutorial data, click Auto Assign.



Note: When using your own data, click Manually Assign if your file names do not conform to the example nomenclature. Then drag-and-drop traces directly into the application to create the Amplicon and Specimen Trace Grid.

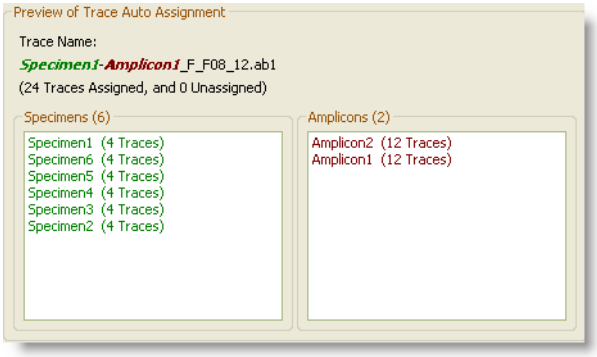
2. Use the drop-down lists to indicate the specimen ID (sample name) from the file string. Select the placement of the specimen name in relation to the first delimiter, then select the delimiter type (period, underscore, or dash).

3. Repeat step 2 to assign the amplicons.



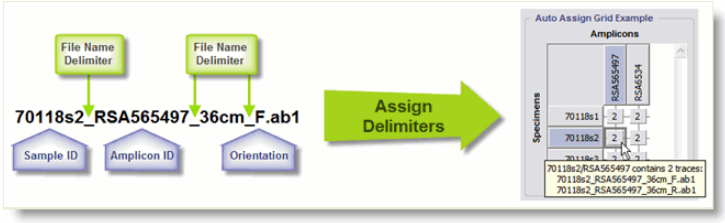
The 24 assigned traces group into 6 specimens and 2 amplicons.

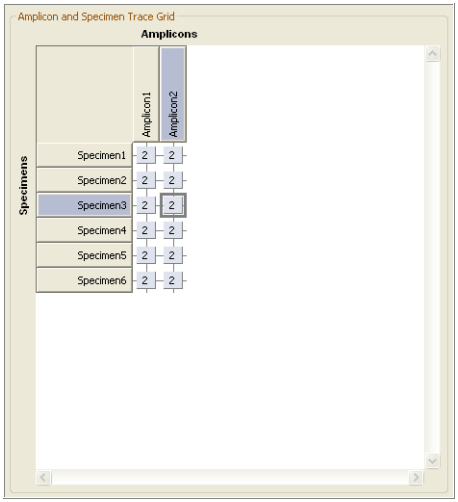
The Preview Results pane will look like this if parsed correctly:



Note: For tutorial data, use the settings shown in steps 2 and 3.

4. Click **OK** to create the Amplicon and Specimen Trace Grid.





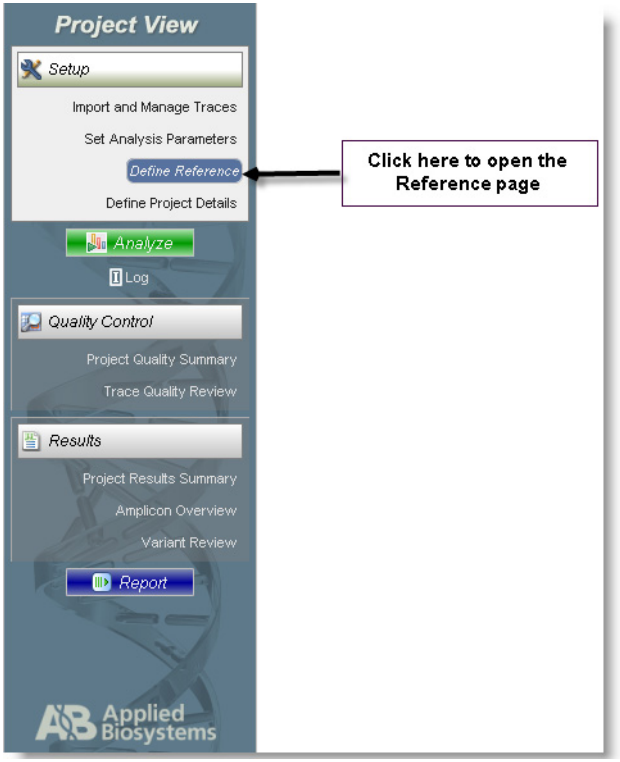
Next, you import a reference for this project.

Specify a reference

Import a reference

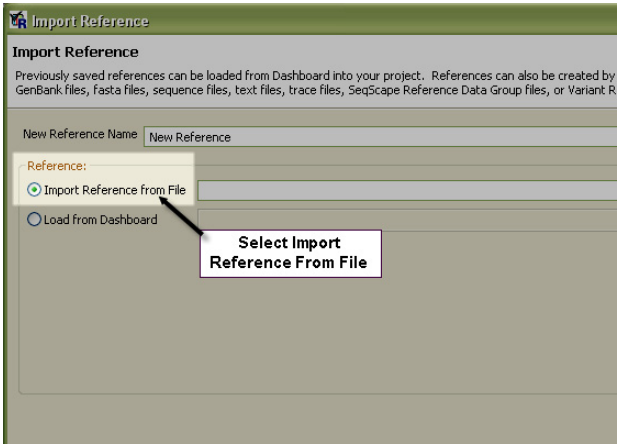
In this section you will import two .fsta files included in the Tutorial Data folder to use as references. Segment 1 is the reference you will use for Amplicon 1 and Segment 2 is the reference you will use for Amplicon 2.

1. In the Task pane, select **Define Reference** to open the Define Reference page.

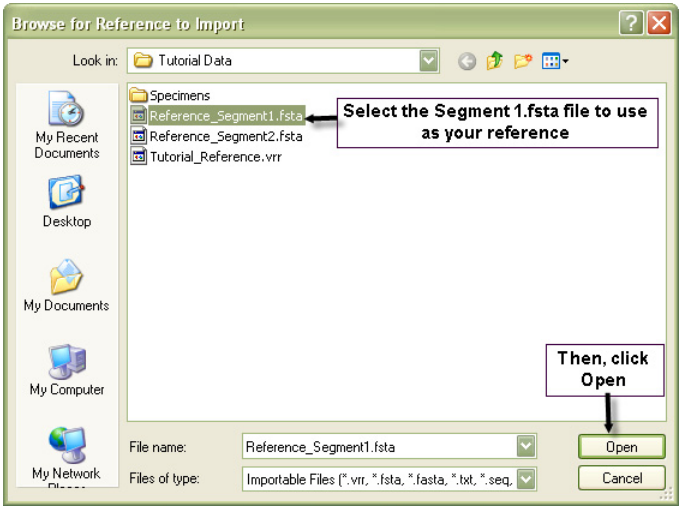


2. Click Import (Task Action toolbar) to import a reference.

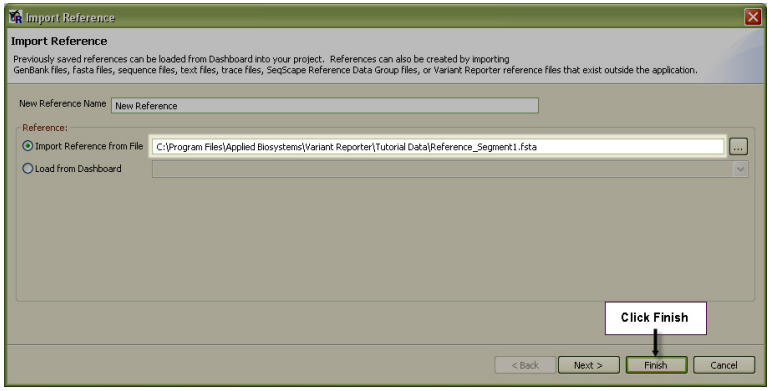
3. In the Import Reference dialog box, select **Import Reference from File**, then browse to the Tutorial Data folder.



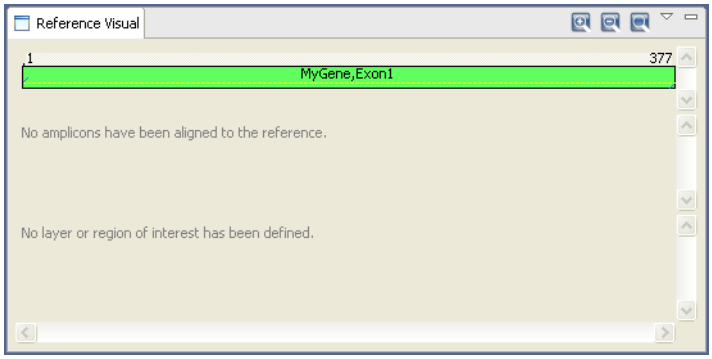
4. Select **Reference_Segment1.fsta**, then click **Open**.



5. Verify the .fsta file in the reference field, then click **Finish**.



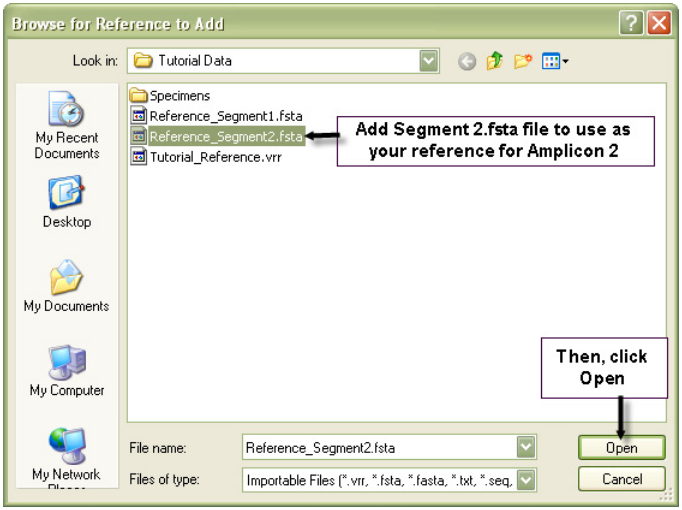
The imported reference displays in the Reference Visual pane.



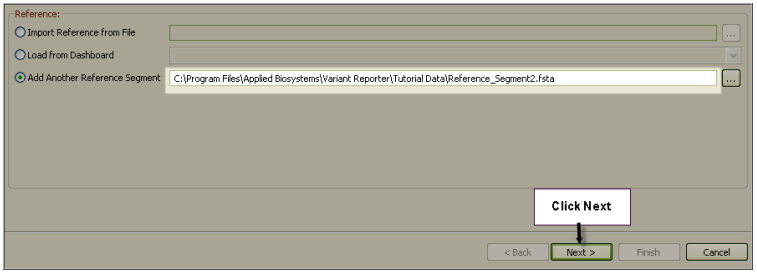
Add another reference segment

To add Segment 2 as the reference for Amplicon 2:

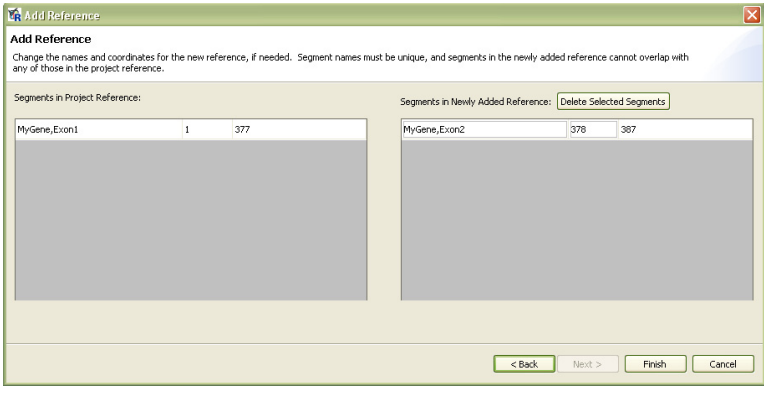
1. In the Define Reference Page, click Import.
2. Select **Add Another Reference Segment**, then click ... to browse back to the Tutorial Data folder.



3. Select **Reference_Segment2.fsta**, then click **Open**.



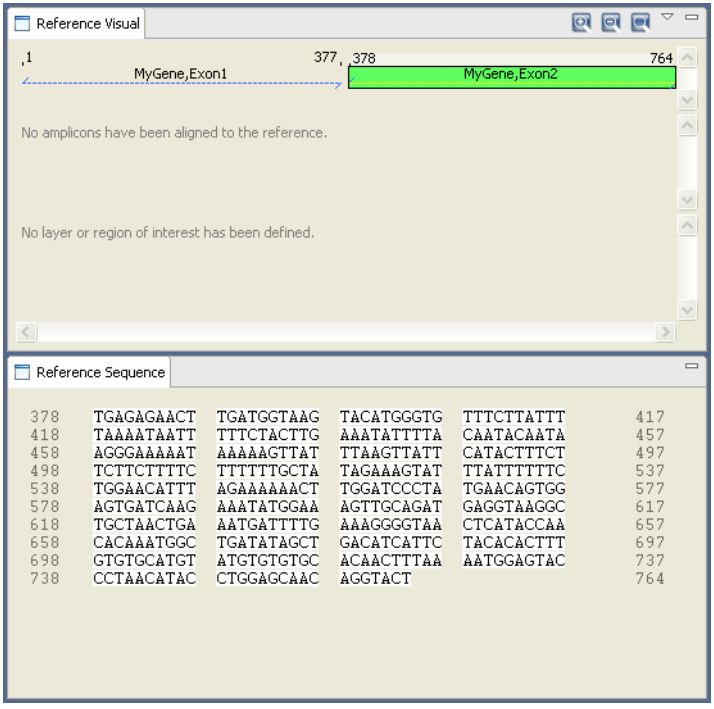
4. Verify the .fsta file path in the reference field, then click **Next**.



5. In the Add Reference dialog box, confirm the new reference aligns to the correct region of the sequence, then click **Finish**.

Note: You can edit the reference name or its coordinates in this window.

The second reference displays in the Reference Visual pane.



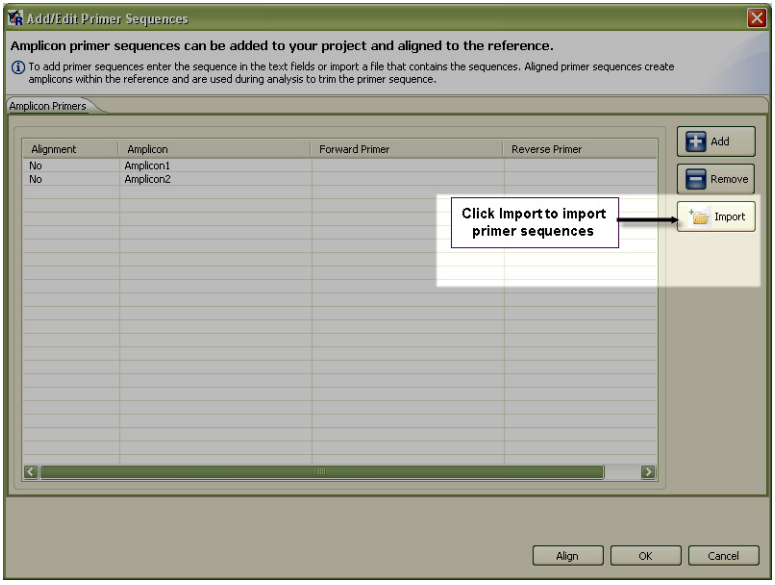
Set up the amplicons

Import the primer file

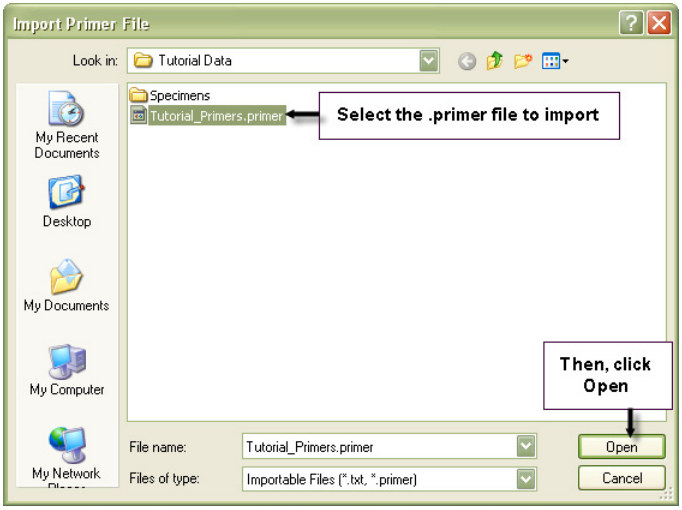
Use the primer file included in the Tutorial Data to set up the amplicons.

1. In the Define Reference page, click Amplicon Primers/Known Variants (Task Action toolbar), then select **Add/Edit Primer Sequences**.

2. Click **Import** to open the Import Primer File dialog box.



3. Select the **Tutorial_Primers.primer** file in the Tutorial Data folder, then click **Open**.

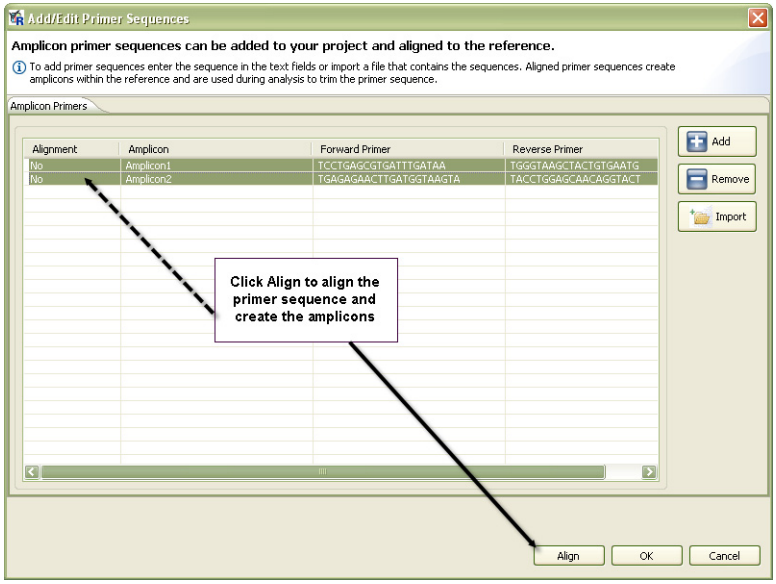


4. Click **Yes** at the warning prompt (replace amplicon names with new primers).

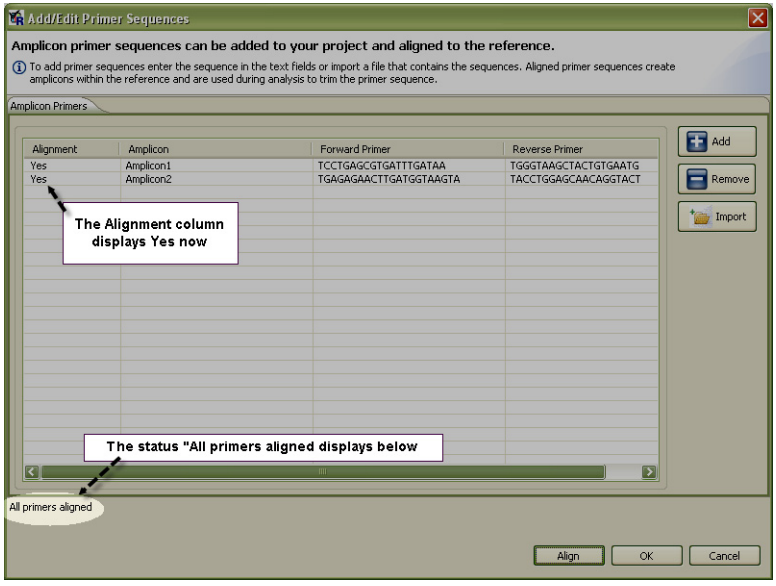
Align the primer to the reference

Next, you will align the amplicons to the reference.

1. Select both **Amplicon 1** and **Amplicon 2**, then click **Align**.



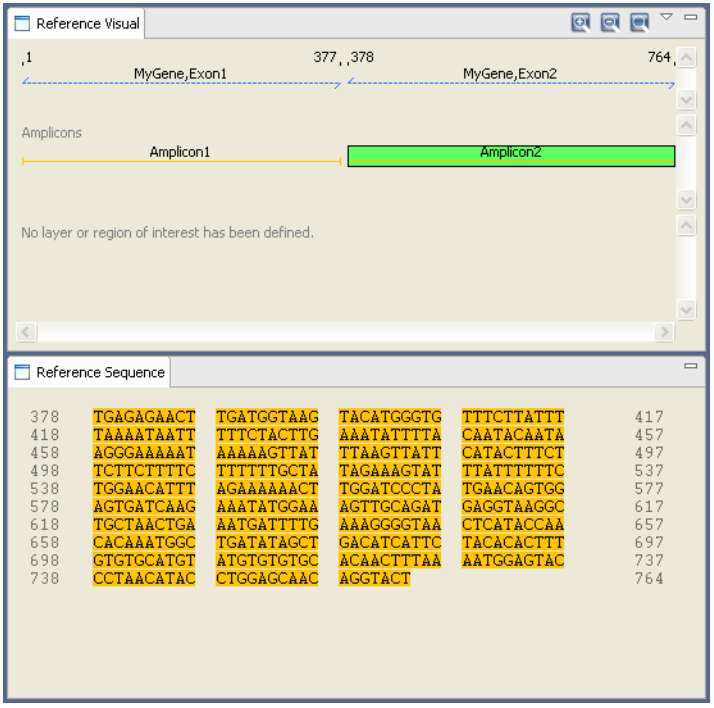
2. Verify the Alignment column displays **Yes**.



IMPORTANT! When the Tutorial amplicons are aligned correctly, the Alignment Status column displays Yes and All primers aligned displays in the lower left-hand corner of the Add/Edit Primer Sequence dialog box. When you are using your own data, note that if the alignment displays No, you have to manually correct the amplicons or check the original file for sequence error.

3. Click **OK**.

4. Verify that the amplicons look like this in the Reference Visual (Define Reference page).



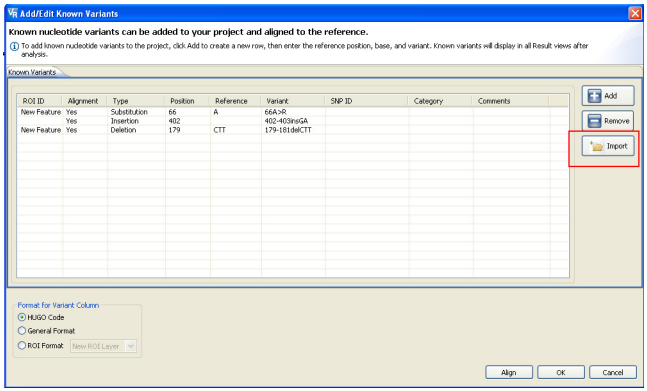
IMPORTANT! The Amplicon name *must* match the Amplicon name in the Amplicon and Specimen Trace Grid. If the names do not match, the amplicons are shown under Unaligned Amplicons in the Define Reference page.

Align known variants to the reference

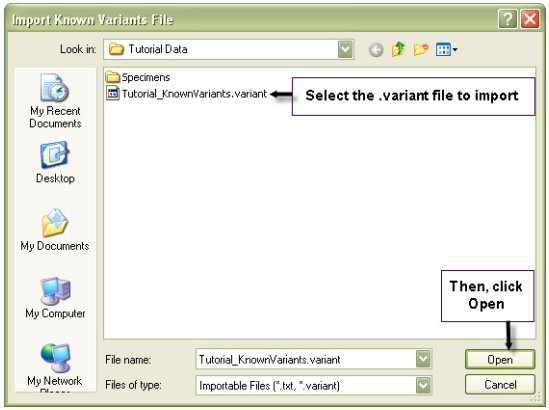
A known variant is a variant with importance or interest to you that you define before analysis while creating a reference sequence.

Import the known variant file included in the Tutorial Data.

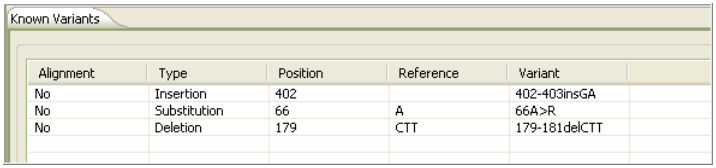
1. In the Define Reference page, click Amplicon Primers/Known Variants (Task Action toolbar), then select **Add/Edit Known Variants**.
2. Click **Import**.



3. Select **Tutorial_KnownVariants.variant**, then click **Open**.



4. Verify that three known variants display in the Add/Edit Known Variants dialog box.



Note: When working with your own data, you can change the format of variants from HUGO (default) to General, if needed.

5. Select all known variants, then click **Align**.

   'Yes' will display in the Alignment column.

6. Click **OK**.

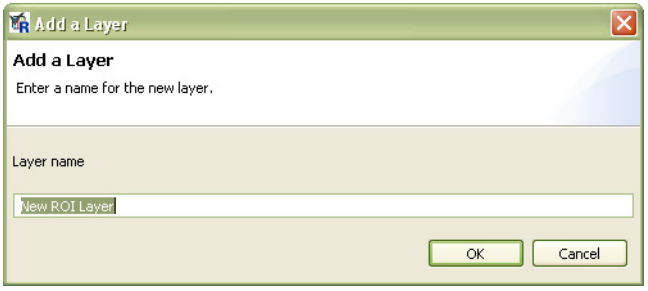
Create layers and regions of interest (ROIs)

Create a layer

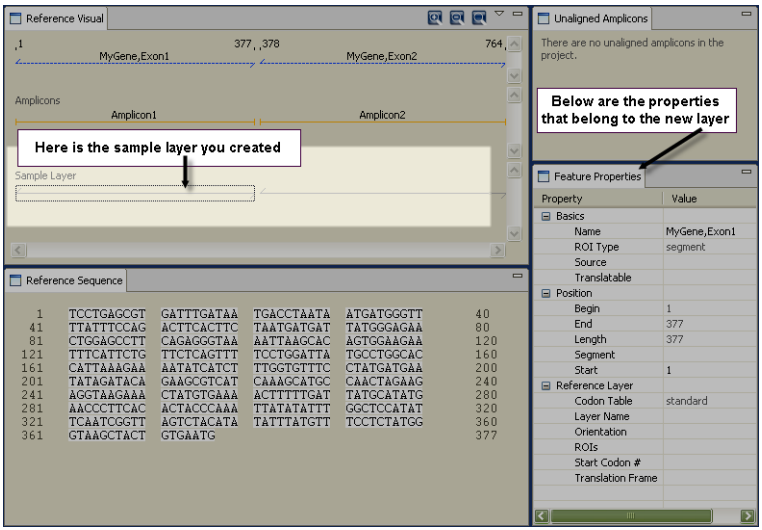
A layer is a group of related, non-overlapping regions of interest (ROIs) that a user can define as part of the reference sequence. A layer can represent a gene that contains properties of orientation, translation frame and codon start number.

1. In the Define Reference page, click Layers (Task Action toolbar), then select **Add Layer**.

2. Name your new layer, then click **OK**.



3. Confirm the new layer in the Reference Visual pane.

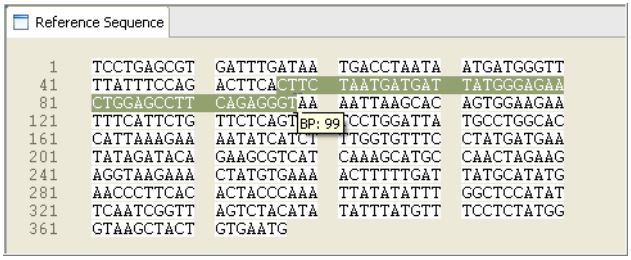


Note: You can change the orientation of a layer in the Feature Properties pane. Select the layer in the Reference Visual, then click the parameter's field that you want changed to make your edit. You can also change the translation frame number or the start codon number associated with the selected layer.

Create a ROI

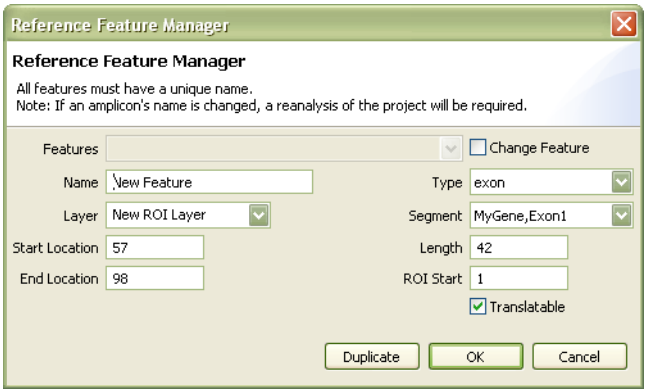
A region of interest (ROI) is the part of the reference sequence that you want to highlight. A ROI could represent an exon, intron, amplicon, or an entire gene.

1. Select a particular segment of the reference (Reference Visual pane) to call out as a ROI by manually highlighting a portion of the sequence. (Reference Sequence pane)



2. Click ROIs (Task Action toolbar), then select **Create/Edit ROI**.

3. Name the new ROI, then select the *type* of region you are highlighting from the drop-down list (exon, intron, gene, amplicon, promoter or generic).



4. Select the layer where you want to associate the ROI, then confirm or change the Start and End Locations and the ROI Start position.

Note: The Start and End Locations refer to the selected sequence start and end points and can be changed in this window.

Note: Check Translatable to be able to see amino acid variants display in the Variant Review summary after analysis.

5. Click **OK**.

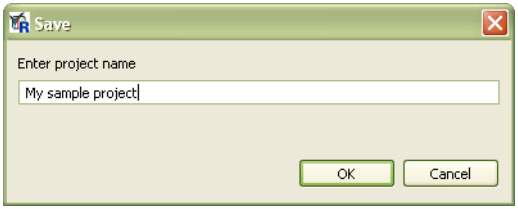
Note: When working with your own data, click Duplicate when you want to create the same ROI on another layer.

6. Click Copy To Dashboard to save the reference to your Data Store for future use.

Save the project

After creating layers and ROIs, save your project.

1. In the Task pane, click **Define Project Details** to open the Project Details page.
2. Type your name in the Created By field.
3. Click Save Project.
4. Enter a name for your project, then click **OK**.



5. Enter your name in the text field, then click **Save Project**.

Analyze the project

Analyze

After setting up a Variant Reporter™ software project by importing and grouping traces into amplicons and specimens, then specifying a reference and creating layers and ROIs, analyze your project.

Click Analyze.

Note: When you are working with your own data, analysis time will vary depending on the number of traces in your project and the specifications of your analysis computer. Refer to "Pre-basecalled data performance" on page 12 to get an estimate of analysis time.

IMPORTANT! When using your own data, note that after Variant Reporter™ software finishes analyzing your project, the resulting page view depends on project quality. If your project met the quality threshold settings, then the Project Results Summary page opens. If your project did *not* meet the quality threshold settings, then the Project Quality Summary page opens for your review.

Reanalyze

Reanalyze a project in Variant Reporter™ software when you:

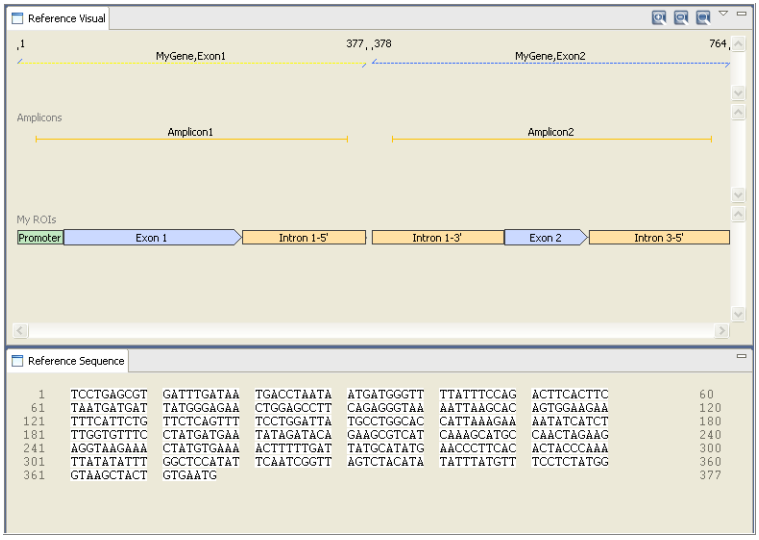
- Update any of the analysis parameters (basecall, trim, or filter)
- Add or delete a reference (including adding or deleting ROIs, layers or amplicons)
- Update the Amplicon Specimen Trace Grid (add, delete, move, unassign, include/exclude individual traces)

When reanalysis of a project is required, the Project Status updates to display 'Reanalysis Required' in the Navigation toolbar and the Analyze button reactivates (displays in bright green).

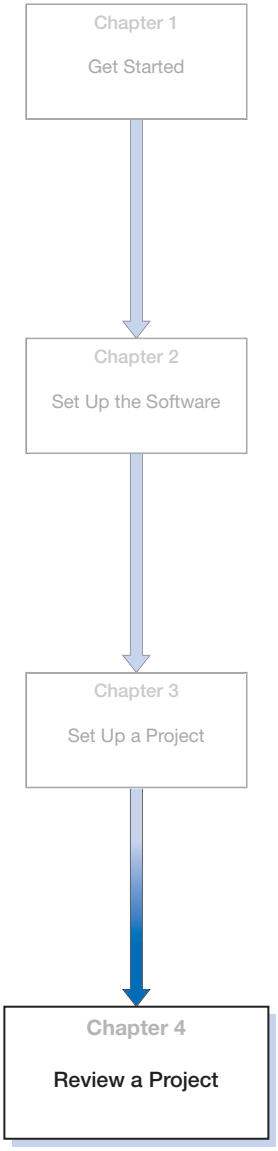
Note: Reanalyzing a project reapplies your base edits and will attempt to reapply your status review edits while auto-updating the variants in the Variant Review page tables.

Sample file with multiple ROIs

As an example, import the Tutorial Data file called **Tutorial_Reference.vrr**. This reference file illustrates a good representation of a project containing a layer with multiple ROIs:



4 Review a Project



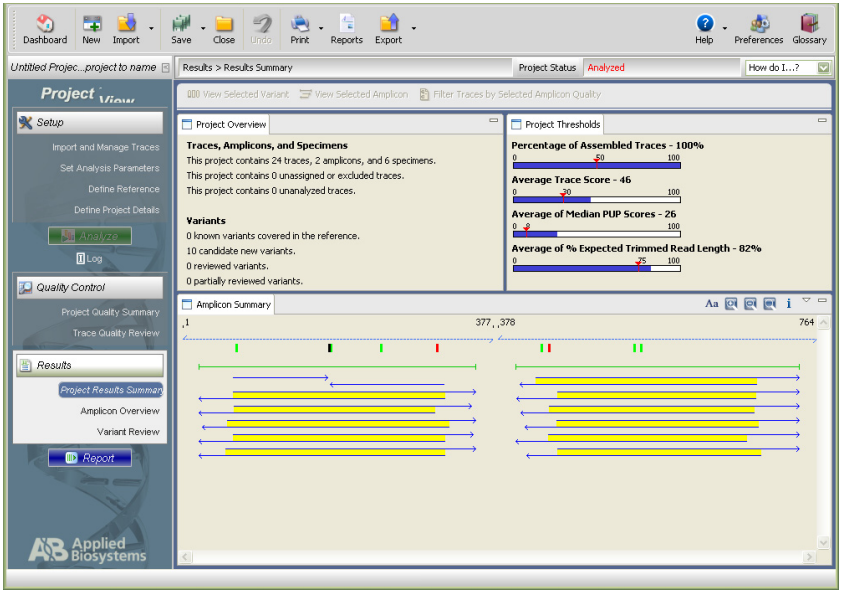
This chapter contains:

- Review project results 40
- Review variants 41
- Edit variants 44
- Report and export project results 45

Review project results

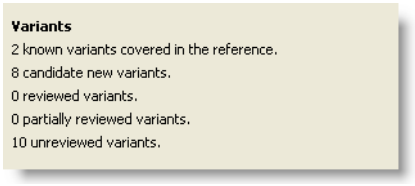
View the Project Results Summary Page

After analysis, Variant Reporter™ Software opens the Project Results Summary page if your project passed the Quality Threshold settings.



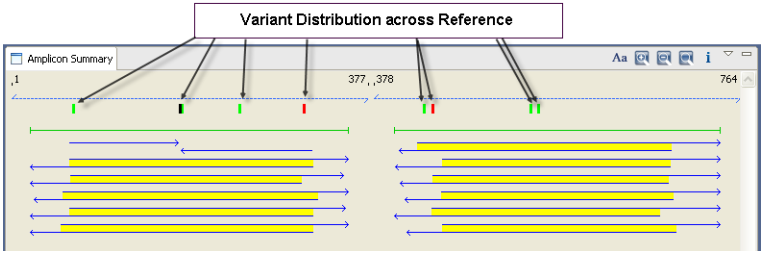
Note: If your project does *not* meet the Quality Threshold settings during analysis, Variant Reporter™ Software opens the Project Quality Summary page, encouraging you to examine where the project failed in detail.

With the tutorial data, you can verify the overall quality of the project in the Project Results Summary page. Check the Variants section in the Project Overview for a consise summary of all variant information.



The Project Results page summarizes:

- number of candidate new variants
- number of known variants
- number of previously reviewed variants
- overview of all amplicons
- variant distribution across the reference



Note: The blue directional arrows represent forward or reverse orientation and the yellow bars represent coverage for each specimen.

Note: Click i to display the Variant legend and the Amplicon Quality legend.

Review variants

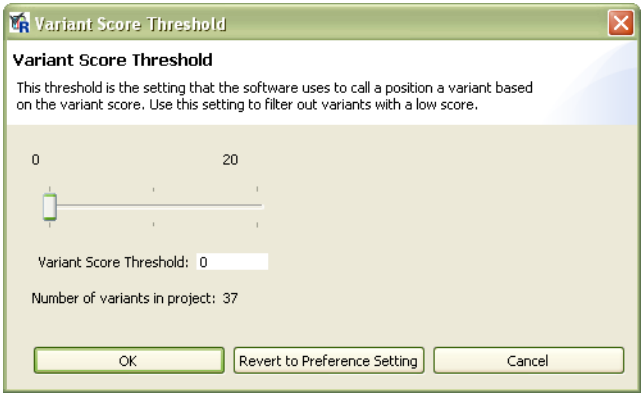
Adjust the Variant Score

Go to the Variant Review page to see a full list of all variants, per amplicon, that the software detected during analysis.

To increase or decrease the sensitivity of false negative or false positive variant calls, adjust the Variant Score Threshold by:

1. Click Variant Score Threshold on the Task Action toolbar.
2. Reset the threshold by dragging the slider between False Positive and False Negative.

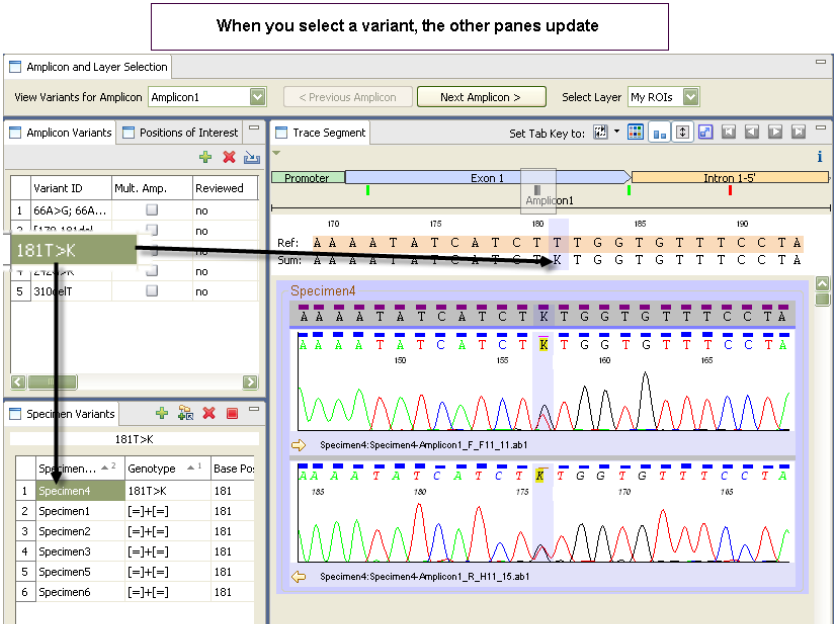
   Note: Moving the threshold setting changes the number of variants to review.



3. Click **OK** after you set the value you want.

Review Specimen Variants

Select the first variant in the Amplicon Variants table; all specimens containing the selected variant highlight immediately in both the Specimen Variants table and the Trace Segment pane.



Note: Click (Pane Action toolbar) to switch to a snippet view in the Trace Segment pane and view multiple trace segments at once.

Accept or Reject Specimens

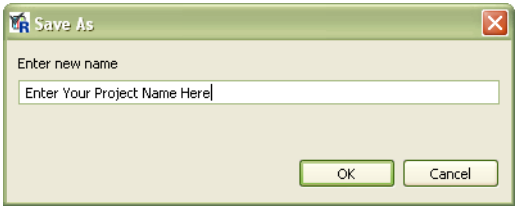
To accept or reject selected specimen genotypes:

1. Select a variant that you want to review in the Variant ID column (Amplicon Variants table).
2. Review the specimens (extended trace view or snippet view) associated with each variant (Specimen Variants table).
3. **Right-click** to accept , or reject , the specimen genotype(s).

   Note: Click to accept or reject all specimen genotypes at once.

   Note: When you accept or reject a specimen genotype, both tables (Specimen Variants table, Amplicon Variants table) update simultaneously.

4. To continue reviewing variants for the next amplicon, click Next Amplicon > at the top of the page.
5. Save your project when you have completely reviewed all detected variants.



Edit variants

Edit Variants in the Specimen Variants Table

To edit variants in the Specimen Variants table:

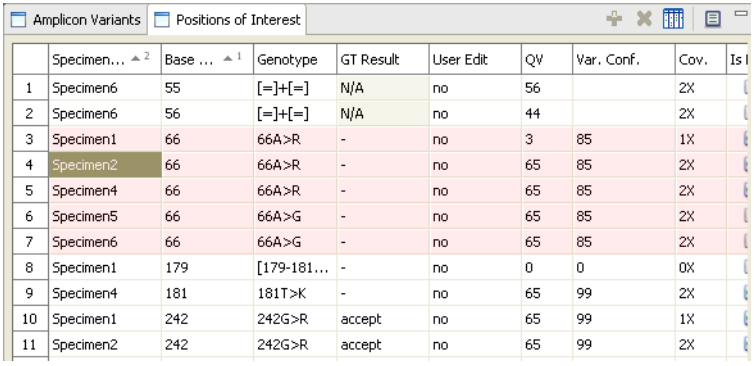
1. In the Genotype column, **right-click** the selected **specimen**.
2. Select one of the following actions:
   - Add Genotype (adds new variant position to the sequence)
   - Change Genotype (assigns different nucleotide)
   - Match Reference (matches reference sequence)

Edit Variants in the POI Table

The Positions of Interest (POI) Table allows you to review a project looking at a specific characteristic at a specific position along the analyzed sequence.

Click  (Pane Action toolbar) to sort the POI Table by:

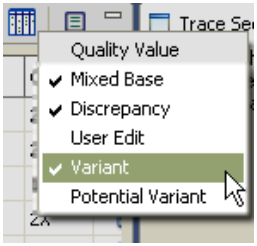
- Quality Values
- Mixed Bases
- Discrepancy
- User Edit
- Variant
- Potential Variant



Amplicon Variants | Positions of Interest

	Specimen...	Base ...	Genotype	GT Result	User Edit	QV	Var. Conf.	Cov.	Is I
1	Specimen6	55	[=]+[=]	N/A	no	56		2X	
2	Specimen6	56	[=]+[=]	N/A	no	44		2X	
3	Specimen1	66	66A>R	-	no	3	85	1X	
4	Specimen2	66	66A>R	-	no	65	85	2X	
5	Specimen4	66	66A>R	-	no	65	85	2X	
6	Specimen5	66	66A>G	-	no	65	85	2X	
7	Specimen6	66	66A>G	-	no	65	85	2X	
8	Specimen1	179	[179-181...	-	no	0	0	0X	
9	Specimen4	181	181T>K	-	no	65	99	2X	
10	Specimen1	242	242G>R	accept	no	65	99	1X	
11	Specimen2	242	242G>R	accept	no	65	99	2X	

Note: You can select multiple project characteristics to review at once.



Report and export project results

Report and Export Results

The Report button is accessible on the Project View page (Task pane) as the last task you can perform in Variant Reporter™ Software.

You can create any of three overall project reports:

- Project Summary Report
- Quality Summary Report
- Specimen Report

You can create any of seven additional trace quality reports:

- QC Report
- Plate Report
- Trace Score Report
- CRL Report
- CRL Distribution Report
- QV20+ Report
- Signal Strength Report

Reports can be previewed, printed, or exported, and multiple reports can be selected at once.

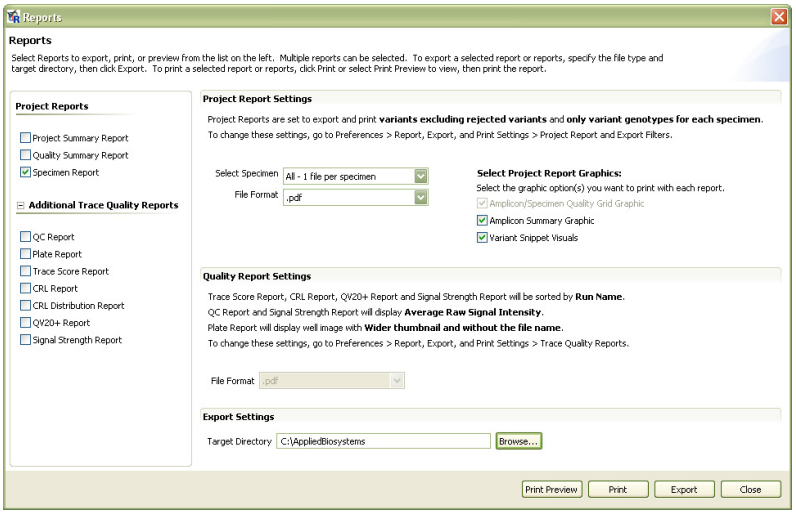
Create a Report

To create a report:

1. Click Report to open the Reports dialog box.
2. Select the checkbox next to the report, or reports, you want to print and/or export.

Note: When selecting a Specimen Report, you must also select the specimen from a drop-down list, then specify the file format.

3. Specify the file format.
4. Specify the target directory you want the report to export to, then click **Print Preview** or **Print**.



Note: The Export feature is available only after you choose a destination path.

Operating the Software from a Command Line

This appendix covers:

- Batch mode operation of Variant Reporter™ software 48
- Commands for projects. 49
- Command details 50

Batch mode operation of Variant Reporter™ software

Overview

This appendix explains how to analyze, assign and export data from the command line interface of the Variant Reporter™ software.

IMPORTANT! Applied Biosystems supports the use of the command line interface only as it is explained in this manual.

Note: If you are unfamiliar with Microsoft DOS, Applied Biosystems recommends running the application from the user interface.

The software operates in batch mode to create, or modify, a project by adding and assigning traces, importing reference sequences and analysis parameters, performing analysis, and exporting results.

Commands must be preceded with a "-" (minus sign) to distinguish them from parameters. Commands are not case sensitive. The software reads commands first, then executes them in the order entered.

Execution

To execute Variant Reporter™ software in batch mode, you must use **vr.bat**. The command line arguments must be preceded by "**batch**."

Example

Assuming that the current directory is not on the path, the following command creates a new project named **myProject** with all its trace files in the directory **C:/mydata.** These traces are assigned to amplicons and specimens based on their file names, then analyzed.

```
.\vr batch  -traces c:/mydata -assign -analyze -
save myProject
```

Commands for projects

The following table contains a list of commands and their functions.

Table 1 Commands affecting the project

Command	Function
analyze	Analyze the project
assign	Assign added traces to an amplicon-specimen pair based on the file name
datastore	Switch data stores
export	Export variant information and consensus sequences
open	Open an existing project
params	Import non-default analysis parameters
reference	Import a reference sequence
save	Save the project
script	Execute commands defined in a file
traces	Add traces to a new or existing project

The following table contains a list of miscellaneous commands and their functions.

Table 2 Miscellaneous commands

Command	Function
help	Show help with a command or a text version of this manual
list	Show a list of commands with their parameters
timing	Turn on timing of command execution
log	Write the console output to a file
backup	Backup the current datastore

Command details

This section describes the usage and description of valid commands.

Command: analyze

`analyze`

Usage

analyze

Description

Analyze the current project.

Command: assign

`assign`

Usage

assign [<rule-code>]

Description

Assign traces to an amplicon/specimen pair based on the file name, where [<rule-code>] is an optional code to describe the parsing rules to use. If the parameter is omitted, the default parsing rules will be used.

The code has eight fields, each consisting of either an occurrence number or a delimiter character. Fields are separated by a colon. The first four fields point to the specimen name, and the second four point to the amplicon name.

Either name may appear first in the file name. All the fields must be present.

Note: A zero value in an occurrence field indicates the start or end of the file name. In this case, the delimiter must still be included, but any valid delimiter can be used. Valid delimiters are '_', '.', and '-' (underscore, period, and minus sign).

Example:

"0:-:1:-:3:-:4:-" means the specimen name starts at the beginning of the file name and ends at the first occurrence of '-'.

The amplicon name begins after the third '-', and ends after the fourth '-'.

0:_:1:_:1:_:2_ recognizes the specimen name N34254 and the amplicon name RS30492 in the file name N34254_RS30492_more_stuff.ab1.

Command: backup

`backup`

Usage

backup <backup-folder-location>

Description

Backs up the current data store, where <backup-folder-location> is the folder where the backup files will be located.

Note: This command has no effect on the currently open project.

Command: datastore

`datastore`

Usage

datastore <data-store-name>

Description

Switches to a new data store, where <data-store-name> is the name of the new data store. This command, if used, must precede all commands that alter the current project.

Command: export

`export`

Usage

export <directory> [<format-code>] <export-code> [<export-code]*

Description

Export tables to files, where <format-code> is one of: CSV, TXT, and <export-code> is one of:

- PV All

  Unique Project Variants
- PG All

  Specimen Genotypes in the Project
- PSC

  Project Summary Consensus Sequences
- SCO All

  Specimen Consensus Sequences in one file
- SCS All

  Specimen Consensus Sequences in separate files

The export and format codes are *not* case sensitive, and the format code is optional.

Note: For variant exports the default is TXT. Sequence files are always in FASTA format (.fsta).

Command: help

`help`

Usage

help [<command>] where <command> is any of the console commands.

Description

To see a complete description of all commands, enter the help command with no parameters. To see a list of the available commands, enter the list command.

Command: list

`list`

Usage

list

Description

Lists the command and parameters for all available commands.

Command: log

`log`

Usage

log <log-file-name>

Description

Logs messages to the file specified by <log-file-name>

Command: open

`open`

Usage

open <project-name>

Description

Open an existing project, where <project-name> is the name that appears in the Variant Reporter™ Dashboard.

Command: params

`params`

Usage

params <analysis-parameters-name>

Description

Import analysis parameters, where <analysis-parameters-name> is the name that appears in the Variant Reporter™ Dashboard.

Command: reference

`reference`

Usage

reference <reference-name>|<reference-file>

Description

Import a reference sequence where <reference-name> is a reference name that appears in the Variant Reporter™ Dashboard, or where <filename> is an external file with one of these extensions:

- fsta
- fasta
- ab1
- gb

Command: save

`save`

Usage

save <project-name>

Description

Save the current project, where <project-name> is the name that will appear in the Variant Reporter™ Dashboard.

Command: script

`script`

Usage

script <script-file> [<substitution-parameter>]

Description

Process commands from a file, one command per line, where <script-file> is the file name and [<substitution-parameter>]* indicates zero or more optional parameters that will replace parameter place holders in the file of the form %1, %2, etc.

Since each command is the beginning of a line, the commands in scripts are not preceded by '-'.

Note: The software preprocesses scripts when it reads the command line. The commands (with substituted parameters) are inserted into the command line, replacing the script command at the time of execution.

Command: timing

`timing`

Usage

timing

Description

Displays the elapsed time in milliseconds to execute each command.

Command: traces

`traces`

Usage

traces <dirName>|<filename> [<dirName>|<filename>]

Description

Import trace files, where <dirName> is the name of a directory containing trace files, or <fileName> is the name of a single trace file. Any number of parameters may be used. Additionally, any number of trace commands may be used.

Note: Files can be added to a new project except when using the `open` command.

Glossary

algorithm	A procedure consisting of a sequence of algebraic formulas and/or logical steps to calculate a value or determine a specific outcome. Algorithms allow processing of large amounts of data such as those produced in sequencing projects.
alignment	The process of lining up two or more genetic sequences to achieve maximal levels of identity for determining the degree of similarity between the sequences.
allele	An alternative form of a gene at a genetic locus; a single allele for each locus is inherited from each parent.
allele frequency	See variant allele frequency.
amino acid	Any of a class of 20 molecules that are combined to form proteins in living things.
amplicon	The segment of DNA that is synthesized using amplification techniques such as PCR.
Amplicon and Specimen Trace Grid	In Variant Reporter™ software, a table matrix within the Import and Manage Traces page where you can assign an unassign traces to amplicons and specimens for your project.
Amplicon Specimen Quality Grid	In Variant Reporter™ software, a table matrix within the Project Quality Summary page that is an overview of the quality status of all traces in your project.
Amplicon Variants Table	In Variant Reporter™ software, an exportable table in the Review Variant page that includes all the variants contained within an amplicon.

analysis	In Variant Reporter™ software, the procedure by which the software algorithms process raw data to yield trace quality results and to detect variants.
Analysis Metrics Table	In Variant Reporter™ software, a table in the Trace Quality Review page that displays the analysis results and quality values.
analysis parameters	In Variant Reporter™ software, the user-defined settings that specify the basecalling, trimming and filtering for the analysis.
assembly	The set of aligned overlapping trace data that results from the sequencing of one PCR product or clone.
average CRL	The average of all the contiguous read lengths for all traces within a project.
average median PUP score	The average of each trace's median peak under peak (PUP) score for all traces that pass analysis. See median PUP score.
average percent expected CRL	The average of each trace's clear range divided by the expected amplicon size for all traces that pass analysis.
average signal	The average raw relative fluorescence signal in relative fluorescent units (rfu) for all dyes across a sequence.
average signal strength	The average base signal for all four dyes across the entire sequence.
average signal to noise	The average relative fluorescence value (in rfu) divided by the noise level for each dye across a trace, that is, the average of the run readings of raw signal strength relative to background noise.
average trace score	In Variant Reporter™ software, the average basecall quality value for all traces that pass analysis.
bases	In DNA, adenine, cytosine, guanine and thymine; in RNA, adenine, cytosine, guanine and uracil. These molecules are called bases because they are alkaline, or basic, in the acidic DNA and RNA structure. They are represented as A, T, C, G and U.

base pair (bp)	Two bases that form a rung of the DNA ladder. Adenine always pairs with thymine and guanine always pairs with cytosine.
base position	A numerical value for a base in the reference.
basecall information table	In Variant Reporter™ software, a table within the Trace Quality Review page that allows the user to see the basecalling settings used during analysis.
basecaller	An algorithm that analyzes chromatogram data in trace files and assigns a base for each peak. *See* KB™ Basecaller.
basecalling	In Variant Reporter™ software, this is the first stage of sequence analysis where the basecalling algorithm analyzes the fluorescence signals collected from the genetic analyzer instrument and returns the sequence of basecalls, quality values, and the electropherogram.
clear range	The region of a sequence that remains after excluding the low-quality or error-prone sequences at the 5 prime and 3 prime ends.
codon	Three contiguous bases in a DNA or RNA sequence that specify a single amino acid.
codon start number	In Variant Reporter™ software, a user can define the first amino acid number which coincides to the number of the first triplet of bases within a layer. See translation.
compact HUGO notation	A simplified version of the HUGO nomenclature that uses the IUPAC codes to represent heterozygous mutations. For example, a heterozygous variant from A to T at position 76 would be described as 76A>W. *See* strict HUGO notation.
consensus sequence	The DNA sequence determined by the sequencing method to be the correct sequence for a specimen over a region of one amplicon or of multiple, overlapping amplicons. *See* specimen consensus.
contig	A contiguous (without gaps) segment of a DNA sequence that has been assembled solely on the basis of direct sequencing information (sequence reads).

contiguous read length (CRL)	The longest uninterrupted segment of bases with quality higher than a specified limit. In evaluating the quality of a base, its quality, and the quality of adjacent bases within a specified window, are used.
CRL Distribution Report	In Variant Reporter™ software, a distribution report that plots the number of traces against the contiguous read length (CRL) distribution. This report provides an overall assessment of the read length for an entire set of imported data.
CRL Report	In Variant Reporter™ software, a trace report that plots the contiguous read length (CRL) distribution. This report can be useful in troubleshooting and easily identifying traces with a low-quality score.
data collection information table	In Variant Reporter™ software, a table within the Trace Quality Review page that allows the user to see relevant instrument-specific data on any selected trace, such as well ID, capillary number, and so on.
Data Store	In Variant Reporter™ software, a folder location that contains all project files, associated trace files, analysis parameters, and reference files. Only projects, references, and analysis parameters contained within the Data Store are viewable in the Dashboard view.
discovered variants	In Variant Reporter™ software, polymorphisms identified by the software algorithm during analysis. *See* known variant.
discrepancy	A instance where the trace consensus differs with the specimen consensus at a specific base position.
downstream	In the direction of a sequential process such as transcription and away from the starting event or location in a process. For example, the coding region is downstream from the initiation codon, toward the 3 prime end of an mRNA molecule.
dye set/primer file	*See* mobility file.
electropherogram	A plot of a fluorescence signal over time; used to derive results from DNA sequencing. Also known as trace.

exclude In Variant Reporter™ software, a user action that removes a trace from analysis but keeps the trace in the project. *See* include.

exon Any region of a gene containing a coding sequence for mRNA, in contrast to introns, or junk DNA, which are removed from mRNA before it is translated into a protein. *See* intron.

export To save data in a format usable by another application program (or instance) and to send the saved data to the other application. In Variant Reporter™ software, you can export:

- Projects, references and analysis parameters (.vrz, .vrr, .vrp)
- Results (.txt or .csv)
- Reports (.pdf, .xls, .html)
- Trace consensi (.annotation, .txt, .jpg, .pdf, .phd.1, .scf, .fsta, .qual, .seq)

extended trace In Variant Reporter™ software, a scrollable graphical trace representation in the Variant Review page that contains the variant and 12 base pairs on either side. *See* snippet.

false positive A result indicating (in error) the presence of a polymorphism where no polymorphism exists.

false negative A result indicating (in error) the absence of a polymorphism where a polymorphism actually exists.

FASTA format A standard text-based file format for storing one or more sequence consensi.

filter parameters In Variant Reporter™ software, user-defined criteria applied during analysis for rejecting traces based on trace score, PUP score and/or percent expected clear read length (CRL).

filtering In Variant Reporter™ software, the process of excluding from analysis traces that do not meet user-defined parameters.

flanking sequence In Variant Reporter™ software, the flanking sequence consists of the nucleotides sequence in the 5' and 3' side of each variant. The 5' and 3' flanking sequences of each variant can be exported within the genotype or specimen results.

flat profile An electropherogram view that displays normalized data as analyzed traces to the average height of peaks in any region across the sequence. *See* true profile.

frameshift mutation A genetic mutation caused by indels, that is, insertion or deletion of a number of nucleotides that is not evenly divisible by three from a DNA sequence. Due to the triplet nature of gene expression by codons, the insertion or deletion can disrupt the reading frame or the grouping of the codons, resulting in a completely different translation from the original.

gap A space introduced into a DNA sequence alignment to compensate for insertions and deletions in one sequence relative to another.

GenBank An NIH genetic sequence database that contains an annotated collection of all publicly available DNA sequences. Part of the International Nucleotide Sequence Collaboration, which is comprised of the DNA DataBank of Japan (DDBJ), the European Molecular Biology Laboratory (EMBL), and GenBank at the National Center for Biotechnology Information.

genotype The genetic constitution of an individual or specimen; the complete set of genes, both dominant and recessive, possessed by a particular cell or organism. In Variant Reporter™ software, the genotype is the allele call at a particular locus.

genotyping The process of determining the genetic variation in an individual.

heterozygous The condition of having two different forms (alleles) of a particular gene, one inherited from each parent.

high-quality values *See* quality values.

HIM A variant that is heterozygous at a specific insertion or deletion site along a sequenced trace. *See* insertion, deletion and heterozygous.

homozygous The condition of having two identical forms (alleles) of a particular gene, one inherited from each parent.

HUGO — Human Genome Organization, an international organization dedicated to the Human Genome Project, specifically tasked with mapping sequencing the human genome.

import — To bring data into one application program from another; in Variant Reporter™ software, you can import:

- Projects, references and analysis parameters (.vrz, .vrr, .vrp)
- Files used to create references (.txt, .fsta, .fasta, .seq, .ab1, .gb, .rdg.ctf)
- Text files containing amplicon primer sequences (.txt, .primer)
- Text files containing known variants (.txt)
- Trace files (.ab1)

include — In Variant Reporter™ software, to designate a trace to be analyzed.

INDEL — A segment of DNA that has been inserted in or deleted from a genome.

insertion — A kind of mutation that is the addition of a DNA sequence into a chromosome.

intron — A segment of DNA (in a gene) that is transcribed (along with exons) into but then removed from the primary gene transcript by RNA splicing to leave mature RNA. *See* exon.

IUB/IUPAC — International Union of Biochemistry/International Union of Pure and Applied Biochemistry.

IUB Mixed Base Code diagram — A tool used to determine which pure bases align with a mixed base letter.

KB™ Basecaller — In Variant Reporter™ software, the algorithm (KB™ Basecaller v1.4.1) that calculates mixed or pure bases and determines sample quality values during analysis.

known variants — In Variant Reporter™ software, polymorphisms that a user defines before analysis while creating a reference sequence. *See* discovered variants.

layer A group of related, non-overlapping regions of interest (ROIs) that a user can define as part of the reference sequence. Could represent a gene that contains properties of orientation, translation frame and codon start number.

locus The position on a chromosome of a gene or other expressed DNA region.

low-quality values *See* quality values.

M13 primer A universal primer sequence typically attached to an amplicon primer for sequencing. Also known as M13 universal sequencing primers.

marker A segment of DNA with a known location on a chromosome whose inheritance can be followed. A marker can be a gene, or a segment of DNA with no known function. Markers are often used as indirect ways of tracking the inheritance pattern of genes that have not yet been identified but whose approximate locations are known.

masking The removal of repeated low-complexity regions from a sequence to improve the sensitivity of sequence similarity searches performed with that sequence. In Variant Reporter™ software, masking involves trimming the M13 primer sequence and the amplicon primer sequence from each trace.

median PUP score The median value within the clear range of the ratio of the signal of the highest secondary peak to the signal of the main called base. *See* PUP score.

missense A point mutation in which a single nucleotide is changed, resulting in a codon that codes for a different amino acid.

mixed base threshold The user-specified analysis parameter setting within basecalling that defines the secondary peak height used to determine when a mixed base is called.

mixed base A base whose identity after analysis is other than a pure base (A, C, G or T). *See* IUB Mixed Base Code diagram.

mobility file	Files that compensate for the electrophoretic mobility differences between the dyes and primers and corrects the color-code according to the chemistry used to label the DNA. Such dye set/primer files are also called mobility files.
NCBI	National Center for Biotechnology Information; a US-based resource for molecular biology information containing public databases of analytical genomic data.
non-coding	Describes variants found in a region of interest which does not contain the instruction to be translated into protein.
orientation	In Variant Reporter™ software, a user-defined setting that specifies the direction forward (right) or reverse (left) in which the layer is translated during analysis.
PCR	Polymerase Chain Reaction; a technique to rapidly amplify predetermined regions of double-stranded DNA using heat-stranded DNA polymerase. Sometimes called molecular photocopying.
peak under peak (PUP)	A measure of noise as calculated as the ratio of the fluorescent signal of the highest secondary peak to the fluorescent signal of the main called base. *See* median PUP score.
PUP score	*See* median PUP score.
percent expected trim read length	The length of the clear range divided by the expected amplicon size for the trace.
percentage of assembled traces	The total number of traces that passed analysis divided by all the included traces within a project.
Plate Report	In Variant Reporter™ software, a trace report that contains thumbnails of raw data displayed in the sequence in which the plate was run. For each trace, quality metrics such as Trace Score and CRL are provided.
polymorphism	A DNA sequence variation. *See* variant.
position of interest (POI)	A segment of DNA that is either low-quality, a mixed base, a discrepancy, or identified as a variant.

Position of Interest Table	In Variant Reporter™ software, an exportable table in the Review Variant page that includes all the positions of interest by amplicon.
Project Genotype Export	In Variant Reporter™ software, a result export that includes all the specimen genotypes within a project.
Project Summary Report	In Variant Reporter™ software, a report that includes the Project Details, Project Statistics, Project Quality Summary, Amplicon Graphic, Variant Table, Genotype Table and Variant Snippets.
Project Variant Export	In Variant Reporter™ software, a result export that includes all the variants in a project.
primer	A nucleic acid strand or a related molecule required as a starting point for DNA replication; because most DNA polymerases can begin synthesizing a new DNA strand only by adding to an existing strand of nucleotides. The length of a primer is usually not more than 50 nucleotides.
pure base	A base whose identity after analysis is an A, C, G, or T.
QC Report	In Variant Reporter™ software, a trace report that provides a summary of the quality of the data. The report contains a histogram for an overview of the distribution of the data, a table of the Traces Score, Contiguous Read Length (CRL), and QV20+ for each trace.
Quality Summary Report	In Variant Reporter™ software, a project quality report that includes the Project Details, Project Quality Summary, Amplicon Graphic, Amplicon Quality Table, Specimen Quality Table, and Trace Review Analysis Metric Table.
quality threshold	In Variant Reporter™ software, the user-defined settings for determining the quality of project, amplicon, or specimen.
quality value	A measure of certainty of the basecalling and consensus calling algorithms; high value corresponding to a low chance of algorithm error. Trace quality values are the per-base quality values for a trace; consensus quality values are per-consensus quality values.
QV20+	The total number of bases in the entire trace that have basecaller quality values equal to or greater than 20.

QV20+ Report — In Variant Reporter™ software, a trace report that graphs a QV20+ count for each trace file. Helpful for troubleshooting and easily identifying traces with low counts of QV20+ bases.

reference sequence — The nucleotide string to which all specimen consensus sequences are compared.

region of interest (ROI) — The part of the reference sequence that the user wants to highlight. An ROI could represent an exon, intron, amplicon, or an entire gene. *See* layer.

remove — In Variant Reporter™ software, the user-specified action that eliminates a trace from the project and deletes the trace file.

resequencing — Sequencing of a previously sequenced site using different samples for polymorphism discovery or other purposes.

reverse complement — The DNA/RNA sequence derived by reading the original base sequence in reverse order and exchanging each nucleotide with that of its complement (A-T, C-G).

ROI type — In Variant Reporter™ software, the type of DNA sequence unit specified for study by a user. The types are:

- Amplicon
- Exon
- Intron
- Gene

run information table — In Variant Reporter™ software, a table within the Trace Quality Review page that displays the instrument run settings as defined by the software or modified by the user.

sample — *See* trace.

segment — A contiguous portion of the reference sequence corresponding to a single contiguous DNA sequence.

sequence — The order of nucleotides in a segment of DNA or RNA.

sequencing Analytical process to determine the order of nucleotides in a DNA or RNA molecule.

signal intensity The average raw relative fluorescence signal in relative fluorescent units (rfu) for each dye across a sequence.

Signal Strength Report In Variant Reporter™ software, a trace report that provides the trend of the Raw Signal/Signal-to-Noise intensity for a project. The colors in this line graph represent the four dye colors of A, C, G, T.

silent Describes variants that do not result in a change of the amino acid.

snippet In Variant Reporter™ software, a graphical representation of the variant site that displays the variant and three base pairs on either side. *See* extended trace.

SNP Single nucleotide polymorphism, the most common form of DNA variation (involving a change) to a single base. Occurs when a single nucleotide in the genome differs between members of its species. SNPs can be used as markers.

SNP ID A reference SNP ID number, or "rs" ID, is an identification tag assigned by NCBI to a group (or cluster) of SNPs that map to an identical location. The rs ID number, or rs tag, is assigned after submission to the dbSNP database. For more information, see: http://www.ncbi.nlm.nih.gov/projects/SNP/.

space character In Variant Reporter™ software, a character in an aligned sequence that is shown as a dash (-), indicating a deleted base or, equivalently, an inserted base in one of the other aligned sequences.

specimen A group of traces from the same biological source.

specimen consensus The output of the consensus-calling algorithm from a biologically-related sample group.

Specimen Genotype Export In Variant Reporter™ software, a result export that contains all genotypes for a specimen.

Specimen Report — In Variant Reporter™ software, a report that includes the following sections based on one specimen: Specimen Details, Specimen Quality Statistics, Specimen Variant Statistics, Amplicon Graphic, Specimen Genotype Table, and Specimen Variant Snippets.

Specimen Variants Table — In Variant Reporter™ software, a table in the Review Variant page that includes all specimen genotypes for a selected variant. The table is exportable as a concentration of all specimen genotypes by amplicon.

strict HUGO notation — A nomenclature system for the description of mutations and polymorphisms that precisely defines the genotype for each allele. For example, [76A>T]+[=] represents a heterozygous mutation from A to T at position 76. *See* compact HUGO notation.

substitution — A type of mutation in which one nucleotide in a DNA sequence is replaced by another nucleotide, or one amino acid in a protein is replaced by another amino acid.

summary sequence — One kind of assembly consisting of all the specimen consensus sequences of an amplicon. If a position within any of the specimen consensus sequences does not match the reference sequence, it is represented as mixed base. Also known as project summary consensus.

thumbnail — In Variant Reporter™ software, a graphical representation within the Trace Quality Review page that displays raw electropherograms in reduced size in a single view.

trace — The output file from a single lane or capillary on a sequencing instrument that is imported into the Variant Reporter™ software.

Trace Identification Table — In Variant Reporter™ software, a table in the Trace Quality Review page that allows the user to see data (such as file name) associated with any selected trace.

trace quality value — *See* quality value.

trace score — The average basecall quality value of bases in the clear range sequence of a trace.

Trace Score Report	In Variant Reporter™ software, a trace report that displays the trace scores for basecalled traces. Helpful for troubleshooting and easily identifying traces with low-quality scores.
translation	In Variant Reporter™ software, a property of the regions of interest (ROIs) that can be turned on or off. If on, the ROI displays as an amino acid sequence.
trimmed read length (TRL)	The length of the sequence that remains after trimming.
trimming	In Variant Reporter™ software, removing before analysis the low-quality data typically found at the beginning and end of a sequence.
true negative	A sequence position that is not detected as a variant and where a variant does not exist.
true positive	A sequence position that is detected as a variant and where a true variant exists.
true profile	An electropherogram view that displays data as analyzed traces scaled uniformly so that the average height of peaks in the region of strongest signal is about equal to a fixed value. *See* flat profile.
unassign	In Variant Reporter™ software, to remove the association of a trace file with an amplicon and specimen.
variant score threshold	A score assigned to polymorphism detection at a sequence position indicating the likelihood that the detection is accurate, for example, SNP confidence value. Higher scores correspond to detections with higher confidence.
variant	In Variant Reporter™ software, a specimen consensus base that differs from the reference sequence. Also known as a polymorphism.
variant allele frequency	The frequency of the variant allele at a polymorphic locus. (VAF)
variant category	In Variant Reporter™ software the variant category can be used to classify known variants in sub-categories such as Polymorphism, SNPs, Insertion, deletion, and CpGs positions.

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