

# Minor Variant Finder Software v1.0

## USER GUIDE

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#### Revision history

Table 1 Revision history of Pub. no. MAN0014835

Revision	Date	Description
A.0	15 January 2016	New document for v1.0 software.

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# Get started

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## Minor Variant Finder Software

The Minor Variant Finder Software is a simple, easy-to-use desktop software designed for the accurate detection and reporting of minor variants (<25% of a major peak) or 50:50 mixtures as found in a germline heterozygous positions by Sanger Sequencing.

By comparing test specimen and control traces, the software generates a noise-minimized electropherogram for confirmation of minor variants in forward and reverse sequences. The software can detect variants (SNPs or SNVs) with a Limit of Detection (LOD) of 5% with high-quality data in amplicons of lengths 150 to 500 bp. LOD is defined as the lowest level at which sensitivity  $\geq 95\%$  and specificity  $\geq 99\%$  within the overlapping region of forward and reverse test and control .ab1 files.

**Note:** LOD was determined using 5% mixtures that were experimentally created with physical mixtures of molecules, and is not based on peak height ratios in electropherograms.



The software also includes an optional NGS confirmation function.

The Minor Variant Finder Software runs in a web browser window, but does not require connection to the internet in order to run. Data is secure on your desktop computer.

## Download and install the software

1. Download the software from [www.thermofisher.com/mvf](http://www.thermofisher.com/mvf).
2. Install the software on a computer that meets the specifications listed in "Computer requirements" on page 7.

## Start the software

Select  **All Programs** **Applied Biosystems** **MVF** **MVF** or double-click  on the Windows™ desktop.

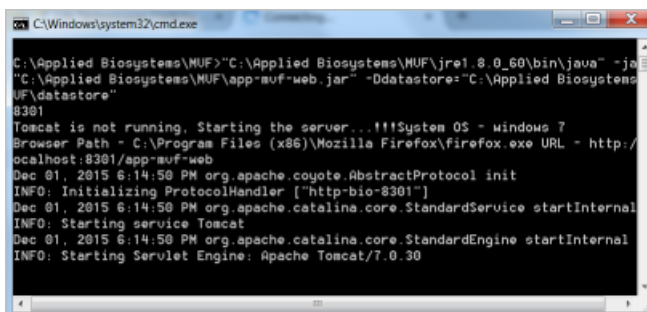
Two windows open:

- An Apache™ Tomcat™ window which must stay open in the background. You do not interact with this window.

---

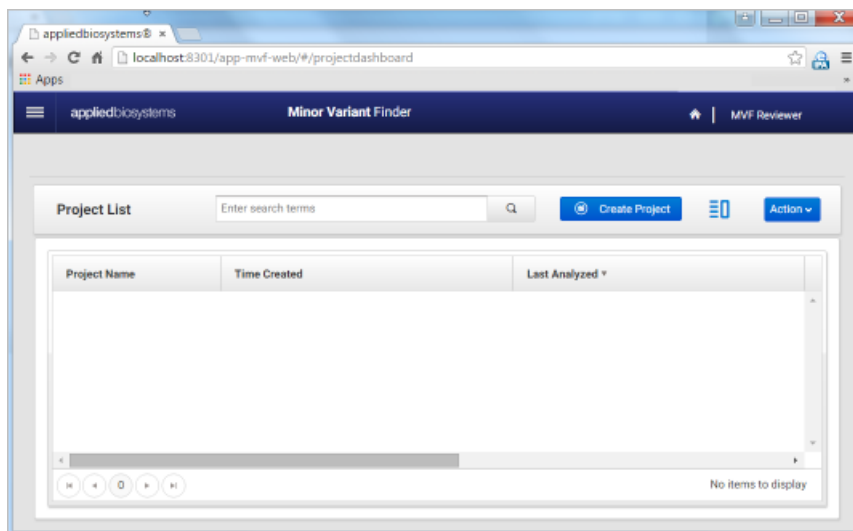
**IMPORTANT!** Do not close this window. If you close the window, the software will not function properly.

---



- A browser window that displays the Minor Variant Finder Software Project screen.

By default, the software opens in Google™ Chrome™, but you can use any of the browsers listed in “Computer requirements” on page 7.





# Requirements

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## Computer requirements

Component	Requirement
Computer	Windows™-compatible computer with 2 GB hard disk space and a minimum of 4 GB memory; 8 GB recommended  Tested configurations: <ul style="list-style-type: none"> <li>Intel™ Core i7-4790 CPU, 3.60 Hz ( Windows™ 10 Pro)</li> <li>Intel™ Core2 DUO CPU E8400, 3.00 GHz (Windows™ 7 SP1) 32-bit</li> <li>Intel™ Core i3-2100 CPU, 3.10 Hz (Windows™ 7 SP1 64-bit)</li> <li>Intel™ Core i5-3380M, 2.9 GHz (Windows™ 7 SP1 64-bit)</li> </ul>
Operating system	Windows™ 7 SP1, 32-bit or 64-bit, or Windows™ 10 Pro, 64-bit
Browser	Google™ Chrome™, Mozilla™ Firefox™, Microsoft™ Internet Explorer™ v.11, or Microsoft™ Edge  <b>Note:</b> The Minor Variant Finder Software runs in a web browser window, but does not require connection to the internet in order to run. Data is secure on your desktop computer.
Screen resolution	1024 x 768 or higher, optimized for 1280 x 1024

## Specimen and control requirements

Requirement	Description
Basecaller	The software can analyze .ab1 files that have been basecalled with KB Basecaller v1.4 or later and that contain sequences that have not been edited with a resequencing software application such as Applied Biosystems™ SeqScape™ Software.
Compatible genetic analyzers	Data from Applied Biosystems™ 3500, 3130, and 3730 Genetic Analyzers has been tested with the Minor Variant Finder Software.  Data from the 3100 models is supported if it is basecalled with the basecaller version stated above.

Requirement	Description
Run conditions	For optimal results, generate the control and test specimen traces at the same time and under the same conditions (the same PCR and sequencing reaction mixes, plates, instrument, and reagents).
Files required for import	<p>To analyze an amplicon, the software requires:</p> <ul style="list-style-type: none"> <li>• One forward and one reverse <i>sample</i> .ab1 file for each specimen</li> <li>• One forward and one reverse <i>control</i> .ab1 file (from a normal control sample with no variants) for the amplicon</li> </ul> <p>The software analyzes these four files simultaneously. The software does not support more than one set of controls per amplicon.</p>
File names	<p>Use the following naming convention for easy grouping of .ab1 files into specimens and amplicons before analysis:</p> <p><i>&lt;SpecimenOrControlName&gt;_&lt;AmpliconName (or Assay ID for Primer Designer™ Tool samples)&gt;_&lt;Orientation&gt;_&lt;WellNo&gt;.ab1</i></p> <p>Important requirements:</p> <ul style="list-style-type: none"> <li>• Include orientation characters "forward", "fwd", or "f" and "reverse", "rev", or "r" (case insensitive) in the file name to represent forward and reverse orientation.</li> <li>• Include one delimiter -_\$.% before and after the orientation characters. The automatic organize function may not work without delimiter characters before and after orientation characters.</li> <li>• Do not include a delimiter character within the part of the file name that represents the amplicon, specimen, or control.</li> </ul> <p>Example of file names that will work properly with the organize function:</p> <ul style="list-style-type: none"> <li>• EGFR_exon_20_BDD-fwd_Control_A01.ab1</li> <li>• EGFR_exon_20_BDD-fwd_Specimen5percent_E01.ab1</li> </ul> <p>Example of file names that will not work properly with the organize function because they contain a delimiter character within the part of the file name that represents the amplicon, specimen, or control:</p> <ul style="list-style-type: none"> <li>• EGFR_exon_20_BDD-fwd_Specimen5%_E01.ab1</li> <li>• EGFR_exon_20_BDD-fwd_Specimen7.5percent_E01.ab1</li> <li>• EGFR_ex.on_20_BDD-fwd_Specimen5percent_E01.ab1</li> </ul>
Sequence orientation	<p>If you will use chromosome position when you create a reference in a project, the orientation of the forward control and the forward test specimen sequences must match the orientation of the reference.</p> <p>See "Check the orientation of sequences in .ab1 files" on page 9.</p>



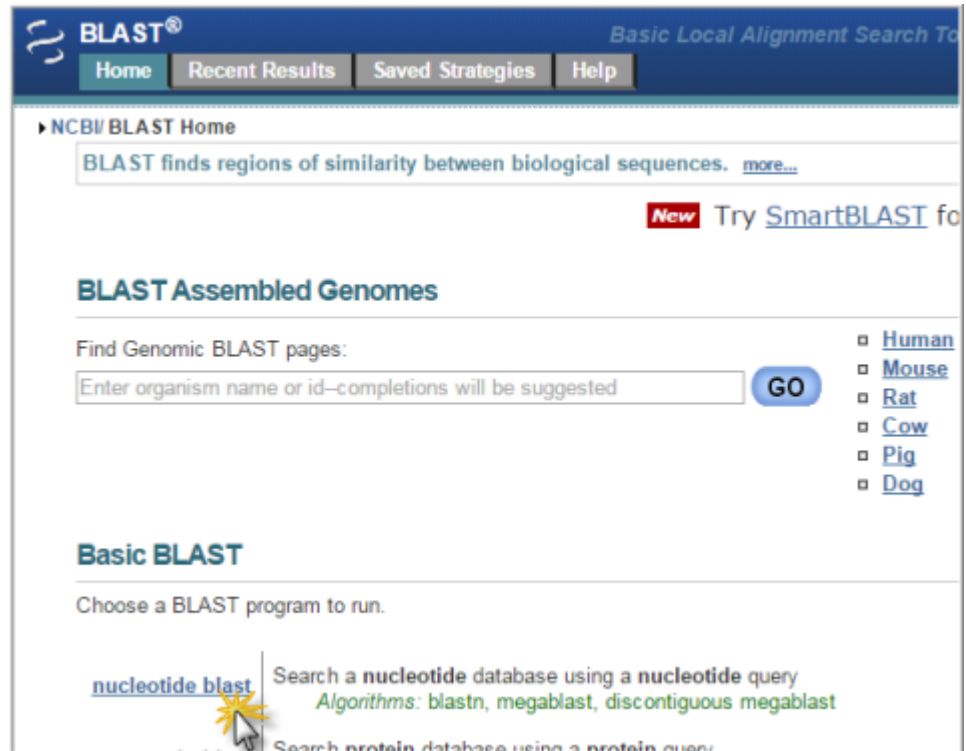
Requirement	Description
Data quality	<p>For optimal results, the software requires high-quality data.</p> <p>See <i>Troubleshooting Sanger sequencing data</i> (Pub. no. MAN0014435) for additional information.</p> <p>If your current operating procedures do not generate the data quality needed, follow the suggested protocols in the following documents to generate high-quality data:</p> <ul style="list-style-type: none"><li>• <i>Generating high-quality data using the BigDye™ Terminator v3.1 Cycle Sequencing Kit</i> (Pub. no. MAN0014628)</li><li>• <i>Generating high-quality data using the BigDye™ Direct Cycle Sequencing Kit</i> (Pub. no. MAN0014436)</li></ul>

## Check the orientation of sequences in .ab1 files

If you will use chromosome position when you create a reference in a project, the orientation of the forward control and the forward test specimen sequences must match the orientation of the reference. To check the sequence orientation, load the sequence of the forward control .ab1 file into BLAST, then compare it to the GRCh37 reference sequence.

1. Create and open a project, then import the forward control trace.
2. Double-click the forward control trace.
3. In the Trace Details screen, click **Sequence**.
4. Select the sequence, right-click, then select **Copy**.

5. Go to [blast.ncbi.nlm.nih.gov/Blast.cgi](http://blast.ncbi.nlm.nih.gov/Blast.cgi), then click **nucleotide blast**.



6. Paste the forward control sequence in the Enter Query Sequence field.
7. In the Database field, select **Genome GRCh37.13**.

8. Click **BLAST**.

The screenshot shows the NCBI BLAST web interface for *Homo sapiens* (human) Nucleotide BLAST. The interface includes a navigation bar with 'Home', 'Recent Results', 'Saved Strategies', and 'Help'. The main heading is 'Homo sapiens (human) Nucleotide BLAST'. Below this, there are tabs for 'blastn', 'blastp', 'blastx', 'tblastn', and 'tblastx'. The 'Enter Query Sequence' section is highlighted with a red box and contains a text area with a FASTA sequence: 

```
Enter accession number(s), gi(s), or FASTA sequence(s)
TGATTGATGCTGTCGCCGGACGATATGAAACAATGGTTCAGTGAAGACCCAGTCCAGATGAAGCTC
CCAGAAATGCCAGAGGCTGCTCCCCCGTGGCCCTGCACCAGCAGCTCCTACACCGCGGCCCTGCA
CCAGCCCCCTCCTGGCCCTGTCTATCTTCTGTCCCTTCCAGAAAACCTACCAGGGCAGCTACGGTTT
CCGCTGGGCTTCTTGCATCTGGGACAGCCAAAGTCTGTGACTTGCACGGTCAGTTGCCCTGAGGGGC
TGGCTCCATGAGACTTCAATGCCTGGCCGATCCCCCTGCATTCTTTTGGTGGAACTTGGGATT
CCTCTGGTCATAGTGTTCCTGA
```

 Below the text area is a 'Browse...' button and the text 'No file selected.'. The 'Job Title' field is empty. The 'Choose Search Set' section is highlighted with a red box and shows a dropdown menu set to 'Genome (GRCh37.p13 reference assembly top-level, Annotation Release 105)' with '(297 sequences)' next to it. Below this are 'Exclude' options, including 'Models (XM/XP)', and an 'Entrez Query' field. The 'Program Selection' section has three radio buttons: 'Highly similar sequences (megablast)' (selected), 'More dissimilar sequences (discontiguous megablast)', and 'Somewhat similar sequences (blastn)'. At the bottom left, the 'BLAST' button is highlighted with a red box. To its right, there is a search bar containing 'Search database Genome (GRCh37.p13 reference assembly top-level, Annotation Release 105) - Homo' and a checkbox for 'Show results in a new window'.

9. Scroll down to the Alignment section and ensure that the Strand result is Plus/Plus, which indicates that the forward control sequence and the NCBI sequence align on their respective forward strands.

**Alignments**

Download ▾ GenBank Graphics

Homo sapiens chromosome 17, GRCh37.p13 Primary Assembly  
Sequence ID: [ref|NC\\_000017.10|](#) Length: 81195210 Number of Matches: 1

Range 1: 7579220 to 7579632 [GenBank](#) [Graphics](#) ▾ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
763 bits(413)	0.0	413/413(100%)	0/413(0%)	Plus/Plus

```

Query 1      TGAGGACCTGGTCCCTCTGACTGCTCTTTTCACCCATCTACAGTCCCCCTGCGGTCCCAA 60
Sbjct 7579632 TGAGGACCTGGTCCCTCTGACTGCTCTTTTCACCCATCTACAGTCCCCCTGCGGTCCCAA 7579573

Query 61     GCAATGGATGATTTGATGCTGTCCCCGGACGATATTGAACAATGGTTCAGTGAAGACCCA 120
Sbjct 7579572 GCAATGGATGATTTGATGCTGTCCCCGGACGATATTGAACAATGGTTCAGTGAAGACCCA 7579513

Query 121    GGTCCAGATGAAGCTCCAGAAATGCCAGAGGCTGCTCCCCCGTGGCCCTGCACCAGCA 180
Sbjct 7579512 GGTCCAGATGAAGCTCCAGAAATGCCAGAGGCTGCTCCCCCGTGGCCCTGCACCAGCA 7579453

Query 181    GCTCCTACACCGGGCCCTGCACCAGCCCTCCTGGCCCTGTGATCTTCTGTCCCT 240
Sbjct 7579452 GCTCCTACACCGGGCCCTGCACCAGCCCTCCTGGCCCTGTGATCTTCTGTCCCT 7579393

Query 241    TCCCAGAAAACCTACCAGGGCAGCTACGGTTTCCGCTCGGGCTTCTTGCAATCTGGGACA 300
Sbjct 7579392 TCCCAGAAAACCTACCAGGGCAGCTACGGTTTCCGCTCGGGCTTCTTGCAATCTGGGACA 7579333

Query 301    GCCAAGTCTGTGACTTGCACGGTCAGTTGCCCTGAGGGGCTGGCTTCCATGAGACTTCAA 360
Sbjct 7579332 GCCAAGTCTGTGACTTGCACGGTCAGTTGCCCTGAGGGGCTGGCTTCCATGAGACTTCAA 7579273

Query 361    TGCCCTGGCCGTATCCCCCTGCATTTCTTTTGTGGAACTTTGGGATTCTCTCT 413
Sbjct 7579272 TGCCCTGGCCGTATCCCCCTGCATTTCTTTTGTGGAACTTTGGGATTCTCTCT 7579220

```

If the Strand result is Plus/Minus, the control and specimen .ab1 files cannot be used with the reference. Rename the files (in Windows™ Explorer) with the opposite orientation. Examples:

- Rename:  
sample ID\_amplicon ABC\_well A1\_ FWD\_.ab1  
to  
sample ID\_amplicon ABC\_well A1\_ REV\_.ab1
- Rename:  
sample ID\_amplicon ABC\_well A1\_ REV\_.ab1  
to  
sample ID\_amplicon ABC\_well A1\_ FWD\_.ab1

# 3

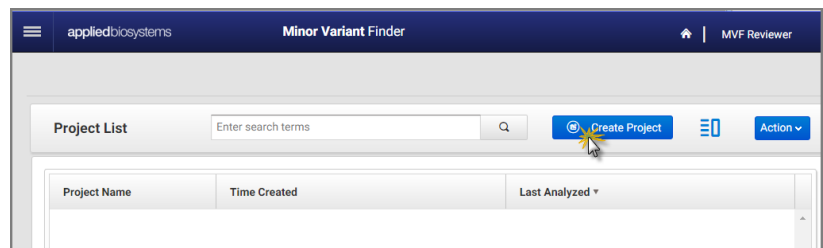
# Use the Minor Variant Finder Software

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## Project workflow

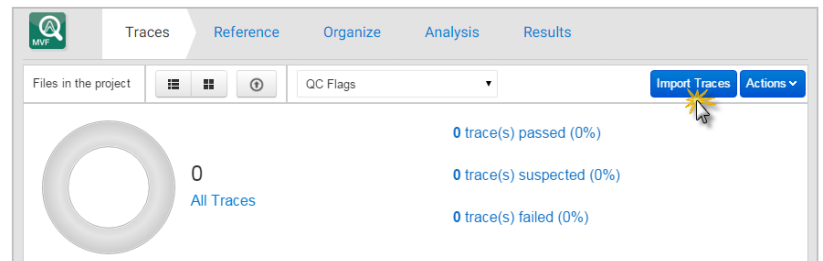
### 1 Project

Create a project

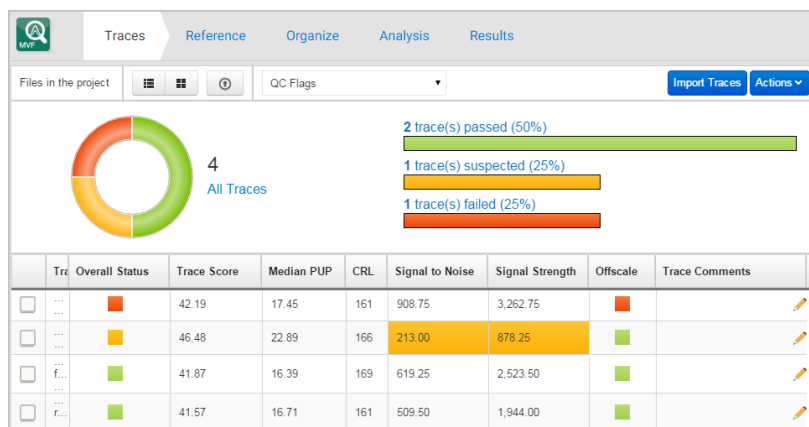


### 2 Traces

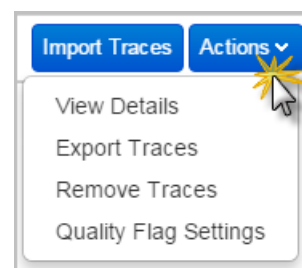
Import sample and control traces



View  
trace  
quality



As needed, view trace details, adjust quality settings, remove low-quality traces



### 3 Reference

For minor variant detection only, without chromosome reference information, click **Use control sequence**, then skip to **4 Organize**.

For minor variant detection with chromosome reference information and/or Next Generation Sequencing confirmation:

1. Select **Create reference sequence**.
2. Specify regions of interest by selecting one of the following (see "Create a new reference" on page 23 for details):

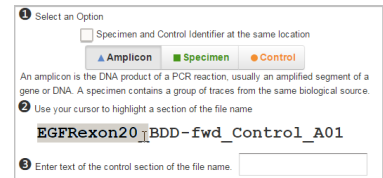
- Import Assay IDs (requires internet connection)
- Enter Chromosome Position (requires internet connection)

**Note:** If you will use chromosome position when you create a reference in a project, the orientation of the forward control and the forward test specimen sequences must match the orientation of the reference.

- Enter a sequence

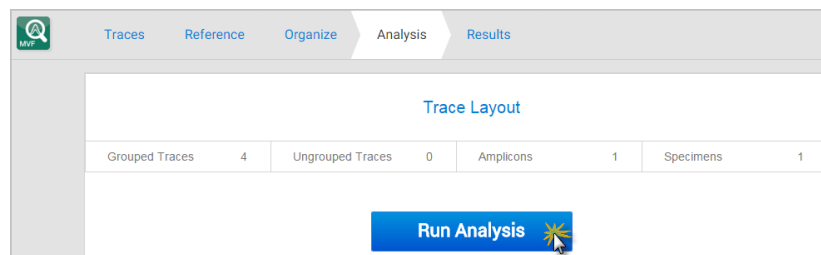
## 4 Organize

1. Click **Organize**.
2. Click **Amplicon**, then select the part of the file name displayed in the dialog box that represents the amplicon.
3. Click **Specimen**, then select the part of the file name displayed in the dialog box that represents the specimen.
4. Click **Control**, then select the part of the file name displayed in the dialog box that represents the control.



## 5 Analysis

Click **Run Analysis**.



**6 Results** see “Results workflow” on page 16.

## Results workflow

Click **Results** in the workflow bar to display the Results screens.

### 1 Summary

Check for analysis errors or warnings. If needed, correct the errors, then analyze again.

See also “How the software handles specimens in which no variants are found” on page 46.

Summary			
Number of Test Specimens:	1	Total number of Variants:	22
Number of Amplicons:	1	Number of Reviewed Variants:	0
Number of Traces Analyzed:	4	Number of Accepted Variants:	0
Number of Unreviewed Variants:	22	Number of Rejected Variants:	0
Number of Partially Reviewed Variants:	0		

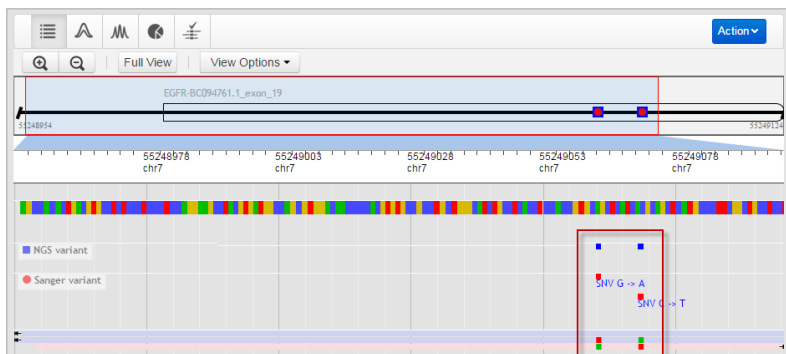
  

Traces with Analysis Errors/Warnings			
Trace Name	Specimen	Amplicon	Errors/Warnings



### 2 Overview

Identify the regions containing variants.



### 3 Variants

Review the variants identified by the software.

Double-click a row to display the electropherograms for a variant.

*(Optional)* Look for non-variants with red review indicators as potential variants, edit as needed (editing a non-variant moves it to the Variants list).

Variants										
Amplicon	Test Specimen	Chromo...	Position	Vari...	Edit...	Review ...	Origin	A...	Review ...	For...
amplicon1	spec1	amplicon1	59	A -> T	N	None	MVF			7.2
amplicon1	spec1	amplicon1	60	C -> T	N	None	MVF			2.8
amplicon1	spec1	amplicon1	89	A -> T	N	None	MVF			8.7



  

Non Variants										
Amplicon	Test Specimen	Chromo...	Position	Vari...	Edit...	Origin	Annotated V...	Review ...	For...	
amplicon1	spec1	amplicon1	88	T ->	N	MVF			NA	
amplicon1	spec1	amplicon1	90	C ->	N	MVF			NA	
amplicon1	spec1	amplicon1	92	G ->	N	MVF			NA	
amplicon1	spec1	amplicon1	108	G ->	N	MVF			NA	



### 4 Electropherogram



1. *(Optional)* “Set the arrow key jump function” on page 39.
2. Review variants in the top noise-minimized forward and reverse traces and review variant peak height percentages. As needed, double-click a variant in the top trace to edit a variant or add a comment. See also “How the software handles multiple variants at the same position” on page 45.
3. Use the center slider bar to zoom on the baseline for all six traces.
4. Type your name in the Reviewer name field at the top right of the screen, then add a comment as needed.
5. Click  to accept or click  to reject the variant.
6. *(Optional)* Select **Actions** ▶ **Generate Report** or **Actions** ▶ **Export Variants**.
7. (Not shown in figure) Press the right or left arrow keys on the keyboard to advance to the next or previous review position in the selected amplicon.



## 5 Summary

Verify that all variants are reviewed.

See also “How the software handles specimens in which no variants are found” on page 46.

Summary			
Number of Test Specimens:	1	Total number of Variants:	22
Number of Amplicons:	1	Number of Reviewed Variants:	0
Number of Traces Analyzed:	4	Number of Accepted Variants:	0
Number of Unreviewed Variants:	22	Number of Rejected Variants:	0
Number of Partially Reviewed Variants:	0		

Traces with Analysis Errors/Warnings			
Trace Name	Specimen	Amplicon	Errors/Warnings



## 6 NGS Confirmation

If you specified a reference and .vcf file in the Reference screen, review Sanger variants that confirm Next Generation Sequencing results.

Chromosome	Position	Annotated Var...	Type	Ref	NGS...	San...	Review Status
7	55248995		Substitution	G	A	-	None
7	55249002		Insertion	C	CCAG...	-	None
7	55249005	rs121913465	Substitution	G	T	-	None
7	55249007	rs147149347	Substitution	G	T	-	None
7	55249010		Substitution	G	A	-	None
7	55249012		Insertion	C	CCGT	-	None




# Create and manage projects

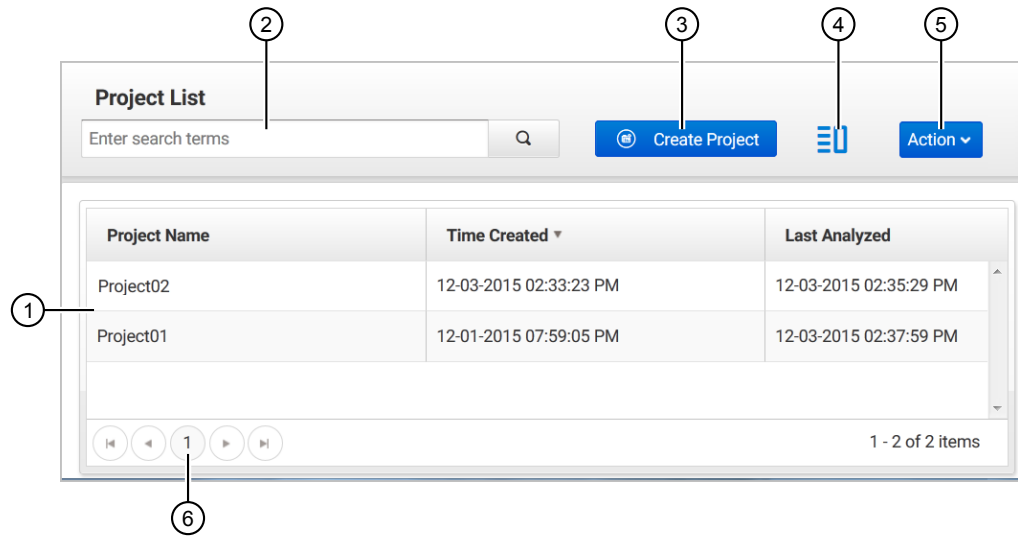
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## Work with projects

In the Project screen:

- Create a project by clicking **Create Project**.
- Open a project by double-clicking it.
- Display project details (metadata) by clicking .
- Import, export, or delete projects by clicking **Action**.  
Exported and imported projects are in .zip format. A project .zip will not import if it has been unzipped then reziped after export.
- Sort projects by clicking table column headers.



## Parts of the Project screen



- ① Project list – Double-click a row to open a project
- ② Search field – Enter project, amplicon, or specimen name
- ③ Create project
- ④ Display project details
- ⑤ Import, export, delete projects
- ⑥ Display additional projects

## Display the Project screen

You can display the Project screen by doing either of the following:

- Click  at the top right of any screen
- Click  at the top left of any screen to close a project



## Import traces

In the Traces screen:

1. Click **Import Traces**.
2. For each amplicon to analyze, select:
  - One forward and one reverse *sample* .ab1 file for each specimen
  - One forward and one reverse *control* .ab1 file (from a normal control sample with no variants) for the amplicon



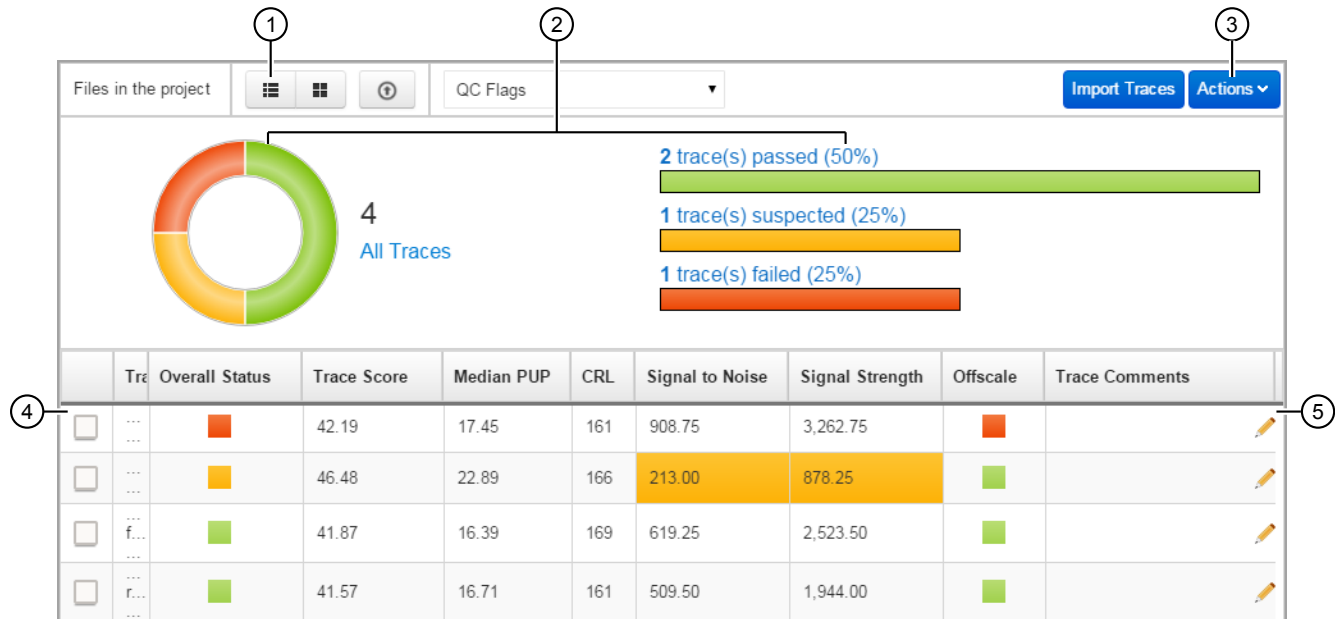
# Traces

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The Traces screen displays information about the trace quality and allows you to remove traces from the project.

Click a color in either the circle or bar quality indicator to display only traces with the selected status color. To display all traces after filtering, click the All Traces link next to the circle indicator.

## Parts of the Traces screen



- ① List and Thumbnail views
- ② Quality indicators – Indicate the pass/suspect/fail status of the traces in the project. You can change the settings for each range (**Actions ▶ Quality Flag Settings**).  
You can click a quality indicator to filter the traces displayed in the table. For example, if you click the green part of the circle or bar indicator, only traces with green status are displayed. To display all traces after filtering, click the All Traces link next to the circle indicator.
- ③ Actions – Click to view trace details, export traces, remove traces from the project, and change QC flag settings.
- ④ Trace selection box – Click to select traces. **Ctrl+click** or **Shift+click** to select multiple traces.
- ⑤ Trace Comment – Click to add a comment. This comment is retained in the Traces screen. It is not included in reports.


For a description of the column headings in the table, select **Actions ▶ Quality Flag Settings**.

## Import traces



In the Traces screen:

1. Click **Import Traces**.
2. For each amplicon to analyze, select:
  - One forward and one reverse *sample* .ab1 file for each specimen
  - One forward and one reverse *control* .ab1 file (from a normal control sample with no variants) for the amplicon

## View analyzed traces and raw data (Trace Details)

1. In the Traces screen, select one or more files.
2. As needed:
  - Click **Actions** ▶ **View Details**, then select **View Markers** and **View Options** to select the attributes to display and set scaling.
  - Click **Raw** or **Analyzed+Raw** to view raw data.  
**Note:** This option is available when only one file is selected in the Traces screen.
  - Zoom.
  - Type a sequence in the Find Sequence box, then click .
3. Click **Close** to return to the Traces screen.

## Change Quality Flag settings

Click **Actions** ▶ **Quality Flag Settings**, then drag  to set the fail (red), suspect (yellow) and pass (green) range for each setting, or double-click , then enter the ranges.

Descriptions of quality settings are displayed on the screen.

## Set view options

1. Access the Trace Details screen (see “View analyzed traces and raw data (Trace Details)” on page 22).
2. Click **View Markers**, then select the bases (low QV, medium QV, N, or mixed) for which to display tick marks at the bottom of the trace pane.
3. Click **View Options**, then select the attributes to display and to set y-axis scaling.

## Scale the y axis in Trace Details

In the Trace Details screen (see “View analyzed traces and raw data (Trace Details)” on page 22):

Select **View Options**, then click .



# Reference

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■ Export a reference file .....	25

The Reference screen allows you to:

- Select the control sequence for viewing in the Results screen if you do not need chromosomal reference information for variants.
- Select or create a reference file for viewing in the Results screen if you need chromosomal reference information for variants. (Variant that are known SNPs are hyperlinked to the dbSNP database.)
- Select a .vcf file if you are confirming next generation sequencing variant results.

If the project already contains a reference file, the information is displayed and you can export it as needed.

If you do not need chromosomal reference information for variants, select **Use control sequence**, then click **Organize** in the workflow bar.

If you need chromosomal reference information for variants or are confirming next generation sequencing variant results, see “Create a new reference” on page 23.

## Create a new reference

If you do not need chromosomal reference information for variants, select **Use control sequence**, then click **Organize** in the workflow bar. You can skip the remainder of this procedure.

If you need chromosomal reference information for variants or you are confirming next generation sequencing variant results, continue with this procedure.

**Note:** If you will use chromosome position when you create a reference in a project, the orientation of the forward control and the forward test specimen sequences must match the orientation of the reference. See “Check the orientation of sequences in .ab1 files” on page 9.

1. Click **Create reference sequence and optionally upload NGS Variants file (vcf)**.
2. Enter a name and description.

3. Select one of the regions of interest options, then click **Save**:

**Note:** The Assay ID and Chromosome Position options require an internet connection. Click  to test the connection.

- **Assay IDs** – (uses Human target GRCh37/hg19 from NCBI)  
Obtain Assay IDs. (For information, see “Obtain Assay IDs” on page 24).  
Click **Choose file** to select the Assay ID file or manually enter or paste the assay IDs (separated by commas).
  - **Enter chromosome position** – (uses Human target GRCh37/hg19 from NCBI)
    - a. Select the chromosome number, then enter a start and stop position. For example, for chromosome 12, you would enter 25378406 for start position and 25378910 for stop position.
  - **Enter sequence** –
    - a. If you will select a .vcf file in the next step, select the chromosome number and enter a start position. For example, for chromosome 12, you would enter 25378406 for start position. If you do not select a chromosome number, the software assigns Chromosome 1. You can overwrite the number by selecting a blank entry from the list.  
If you will not select a .vcf file in the next step, chromosome number and start position are optional.
    - b. Enter a sequence.
4. (Optional for NGS confirmation only) Select a .vcf file containing NGS variants, then click **Save**.
  5. Review the summary, then click:
    - **Organize** in the workflow bar to organize traces.
    - **Actions** ▶ **Export Reference** to export the reference.
    - **Re-create** to discard the reference and create again.

## Obtain Assay IDs

Assay IDs are needed if you want to create a reference using Assay IDs.

Obtain the Assay IDs from any of the following sources:

- Assay shipment packing list.
- Email: Go to the Primer Designer™ Tool  
<http://www.thermofisher.com/primerdesigner>, then email the sequences to yourself (sender is Internet Catalog Orders, format is .csv).
- Export: Go to the Primer Designer™ Tool  
<http://www.thermofisher.com/primerdesigner>, then export the sequences (format is .txt).



## Export a reference file

1. Click **Reference** in the workflow bar.
2. Click **Actions** ▶ **Export Reference**, then select **FASTA** and/or **GenBank** format.



# Organize

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The Organize screen allows you to identify the control traces and to group traces into amplicons and specimens. You can organize traces:

- Manually by dragging traces
- Automatically by clicking **Organize**, then identifying the parts of the file names that represent amplicon, specimen, and control information.

---

**IMPORTANT!** If you change amplicons and specimens in an analyzed project, re-analyze before viewing results. Only the latest results are saved.

---

## Parts of the Organize screen

Click **Organize** in the workflow bar to display the Organize screen.

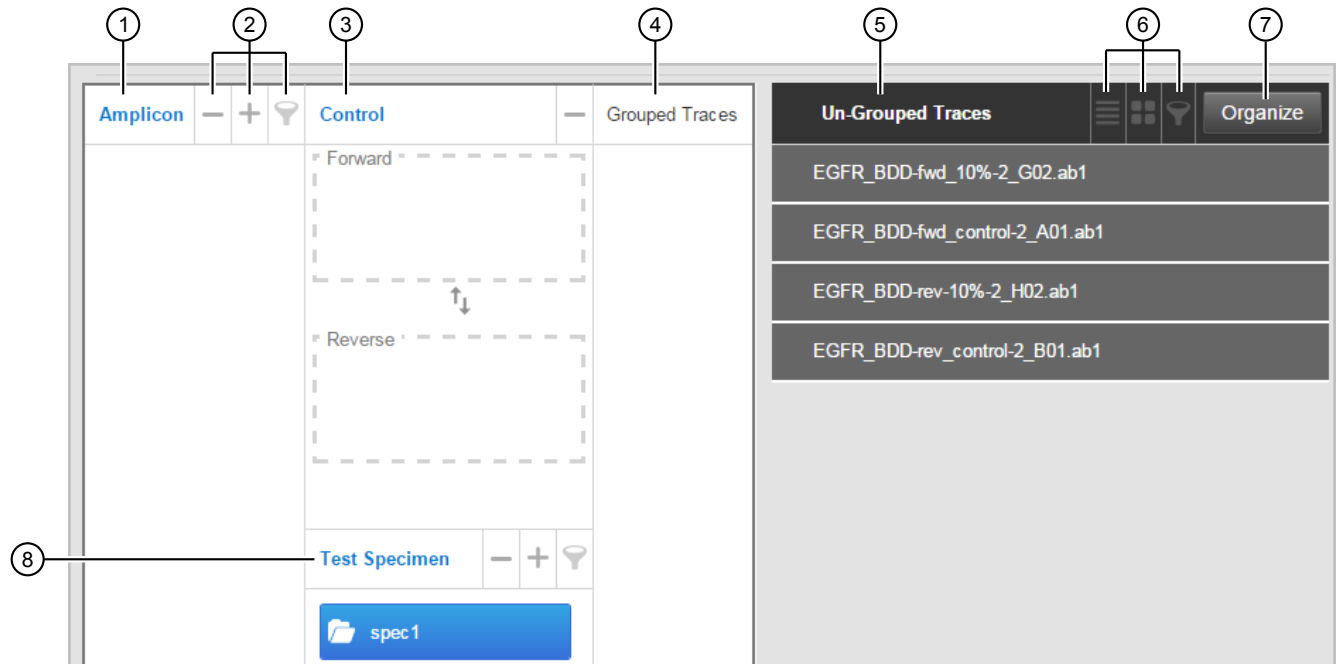




Figure 1 Organize screen

- ① Amplicon – If you specified a reference in the Reference screen, the amplicon name from the reference file is displayed. Click  to manually add an amplicon. Double-click an amplicon to rename it.
- ② Delete, Add, Filter
- ③ Controls
- ④ Traces that have been grouped into the selected amplicon folder
- ⑤ Traces that have not been grouped into an amplicon folder
- ⑥ List view, Grid view, Filter
- ⑦ Organize – Click to use the automatic organize function (see Figure 2). Traces that do not follow the naming convention of the first selected trace in the list are not grouped. You can drag traces to manually group them.
- ⑧ Specimen name – Click  to manually add a specimen. Double-click a specimen to rename it.

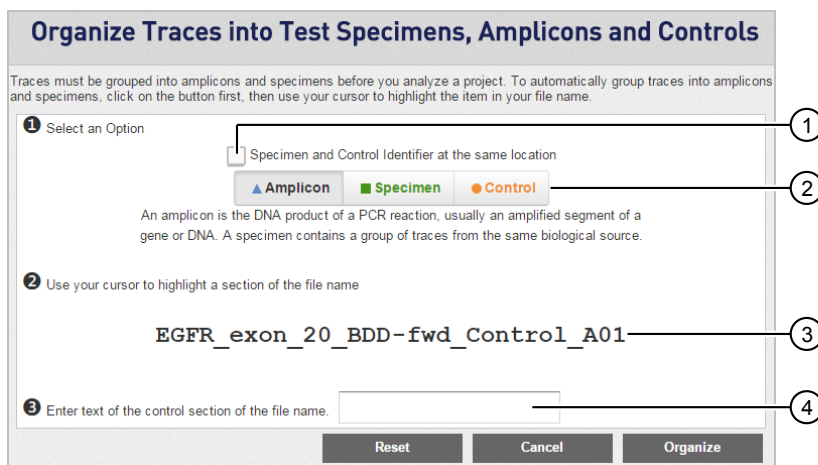


Figure 2 Organize dialog box

- ① Check box to select if your file naming convention uses the same location in the file name to represent specimen and control information
- ② Buttons you click before you select the part of the file name that represents amplicon, specimen, or control
- ③ Name of the first file in the Un-Grouped traces list
- ④ Field where you type the part of the file name that represents the control

## Organize traces automatically

---

**IMPORTANT!** If you change amplicons, specimens, controls, or traces in an analyzed project, re-analyze before viewing results. Only the latest results are saved.

---

The automatic organize function allows you to identify the parts of a file name that correspond to amplicon, specimen, and controls. It then automatically groups traces into control boxes, amplicon folders, and specimen folders.

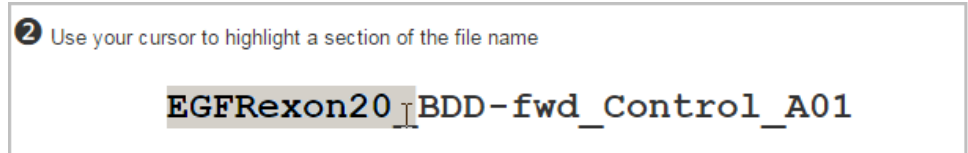
Requirements for automatic organization:

- The name of the amplicon folder must match the part of the file name that you identify as amplicon.
- File names must contain "forward", "fwd", or "f" and "reverse", "rev", or "r" (case insensitive) characters preceded and followed by any of these delimiters -\_\$.%.
- Two control traces can be added to an amplicon folder.
- Two specimen traces can be added to a specimen folder.

1. Click **Organize**.
2. If your file naming convention uses the same location in the file name to represent specimen and control information, select the **Specimen and Control identifier at the same location** checkbox.

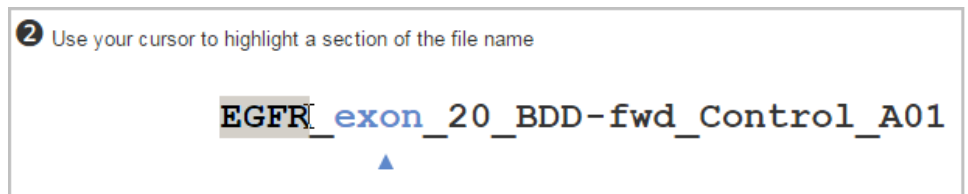
- Click **Amplicon**, then select the part of the file name displayed in the dialog box that represents the amplicon.

Example:



- (Skip this step if you selected Specimen and Control identifier at the same location) Click **Specimen**, then select the part of the file name displayed in the dialog box that represents the specimen.

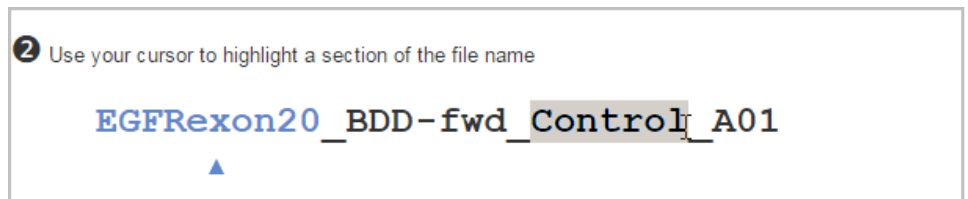
Example:



- If your file naming convention uses the same location in the file name to represent specimen and control information, enter the text of the control section of the file name in the text box.

If your file naming convention uses different locations in the file name to represent specimen and control information, click **Control**, then select the part of the file name displayed in the dialog box that represents the control.

Example:

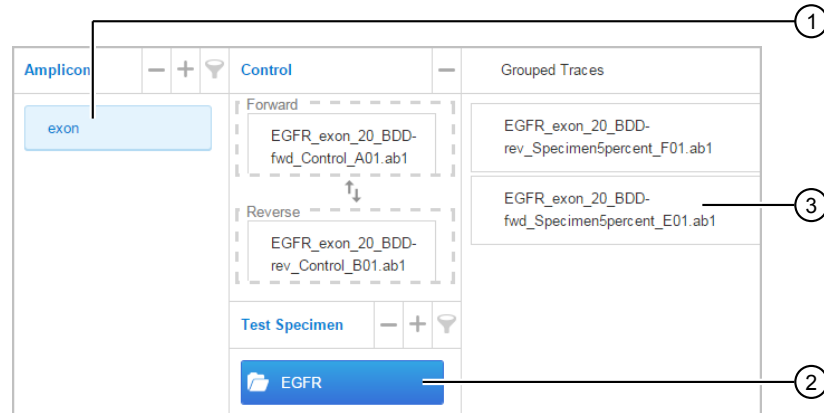


- Click **Organize**.



The dialog box closes and the Organize screen is displayed. The software places controls in the control boxes, automatically creates folders for amplicons and specimens, and groups the corresponding traces into the amplicon and specimen folders.

You can click on the amplicon and specimen folders to list the corresponding traces in the Grouped Traces list.


Example:



- ① Amplicon
- ② Specimen selected in amplicon
- ③ Traces grouped under specimen

7. If any traces remain in the Un-Grouped Traces list, create or select the amplicon and specimen folder for the traces, then click the trace to select it. When it changes to blue, drag it to the Grouped Traces list.
8. As needed:
  - Double-click the Amplicon or Specimen name to edit.
  - Click  to filter amplicons and/or specimens.
  - Click  to remove amplicons and/or specimens.

---

**IMPORTANT!** Before removing amplicons and/or specimens or assigning controls to amplicons, unfilter the list (click , then delete the filter text).



---

## Organize traces manually

---

**IMPORTANT!** If you change amplicons, specimens, controls, or traces in an analyzed project, re-analyze before viewing results. Only the latest results are saved.

---

1. Add amplicon name(s) (if an amplicon name is already listed, skip this step): Click  under the Amplicon header, type an amplicon name, then press **Enter**.
2. Add test specimen name(s) for each amplicon: Select the amplicon folder, then click  under the Test Specimen header, type a specimen name, then press **Enter**.
3. For the control traces: Click a control trace in the Un-Grouped Traces list to select it. When it changes to blue, drag it to the appropriate Control box in the Control column.

If the Control box turns red, it indicates that there is no amplicon specified. Add an amplicon, then repeat this step.

4. For specimen traces: Click one forward and one reverse specimen trace in the Un-Grouped Traces list to select them. When the two trace names change to blue, drag them to the Grouped Traces list.  
Each specimen folder must contain one forward and one reverse trace. If you have replicates for a specimen, create additional specimen folders.  
Repeat for each specimen, one specimen at a time.
5. If needed, add or select another amplicon, then select and drag the control traces and specimen traces to the Control boxes or the Grouped Traces list, respectively.
6. As needed:
  - Double-click Amplicon or Specimen name to edit.
  - Click  to filter amplicons and/or specimens.
  - Click  to remove amplicons and/or specimens.

---

**IMPORTANT!** Before removing amplicons and/or specimens or assigning controls to amplicons, unfilter the list (click , then delete the filter text).

---
7. Click Analysis in the workflow bar.

## Ungroup traces

- To ungroup all traces – Delete all amplicon folders.
- To ungroup individual traces – Click a trace to select it. When it turns blue, drag it to the Un-Grouped Traces list.



# Analysis

Click **Run Analysis** or **Re-Analyze**.






During analysis, the software evaluates:

- The two control traces and two specimen traces at the same time.
- Evaluates the bases and quality values assigned by the KB Basecaller v1.4 or later (.ab1 files must be basecalled with KB Basecaller v1.4 or later before analysis in the Minor Variant Finder Software).
- Compares the forward and reverse specimen traces to the forward and reverse control traces for an amplicon. Analysis requires that at least 15 bases overlap. Variants outside the region where the four traces overlap are not identified.
- Generates a noise-minimized electropherogram and confirms the presence of minor variants on both forward and reverse strands.
- Identifies variants in the region where the forward and reverse test specimen traces overlap with the forward and reverse control traces.
- Assigns review indicator colors (see “Results Review Indicator overview” on page 37).



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The Results screen provides five views:

-  **Overview** – Shows sequence and variant information.
-  **Variants** – Lists the variants found. You can click on known variant names to link to NCBI information on the variant. Also lists low-quality non-variants for evaluation.
-  **Electropherogram** – Displays noise-minimized traces, sample traces, and control traces.
-  **Summary** – Shows variant summary information and analysis error and warning messages.
-  **NGS Confirmation** – Shows the confirmed variants.

If you modify reference, amplicon, specimen, or remove/add trace files after you generate results, click **Re-Analyze** in the Analysis screen to display updated results.

You can generate reports or export variants from any of the views. PDF reports and .vcf/.csv variant export files are saved in the download location specified in the browser.

If a report does not download, see Appendix A, “Troubleshooting”.

# Results workflow

Click **Results** in the workflow bar to display the Results screens.

## 1 Summary

Check for analysis errors or warnings. If needed, correct the errors, then analyze again.

See also “How the software handles specimens in which no variants are found” on page 46.

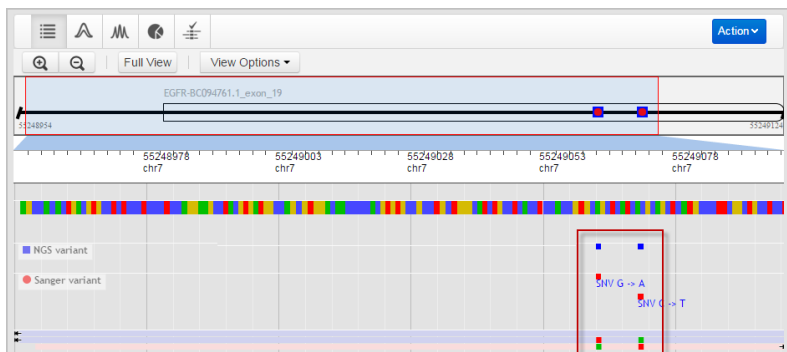
Summary			
Number of Test Specimens:	1	Total number of Variants:	22
Number of Amplicons:	1	Number of Reviewed Variants:	0
Number of Traces Analyzed:	4	Number of Accepted Variants:	0
Number of Unreviewed Variants:	22	Number of Rejected Variants:	0
Number of Partially Reviewed Variants:	0		

Traces with Analysis Errors/Warnings			
Trace Name	Specimen	Amplicon	Errors/Warnings

## 2 Overview

Identify the regions containing variants.



## 3 Variants

Review the variants identified by the software.

Double-click a row to display the electropherograms for a variant.



*(Optional)* Look for non-variants with red review indicators as potential variants, edit as needed (editing a non-variant moves it to the Variants list).

Variants										
Amplicon	Test Specimen	Chromo...	Position	Vari...	Edit...	Review ...	Origin	A...	Review ...	For...
amplicon1	spec1	amplicon1	59	A -> T	N	None	MVF		<span style="background-color: green; color: white;">Review</span>	7.2
amplicon1	spec1	amplicon1	60	C -> T	N	None	MVF		<span style="background-color: yellow; color: black;">Review</span>	2.8
amplicon1	spec1	amplicon1	89	A -> T	N	None	MVF		<span style="background-color: yellow; color: black;">Review</span>	8.7

Non Variants										
Amplicon	Test Specimen	Chromo...	Position	Vari...	Edit...	Origin	Annotated V...	Review ...	For...	
amplicon1	spec1	amplicon1	88	T ->	N	MVF		<span style="background-color: red; color: white;">Review</span>	NA	
amplicon1	spec1	amplicon1	90	C ->	N	MVF		<span style="background-color: red; color: white;">Review</span>	NA	
amplicon1	spec1	amplicon1	92	G ->	N	MVF		<span style="background-color: red; color: white;">Review</span>	NA	
amplicon1	spec1	amplicon1	108	G ->	N	MVF		<span style="background-color: red; color: white;">Review</span>	NA	

## 4 Electropherogram

1. *(Optional)* “Set the arrow key jump function” on page 39.
2. Review variants in the top noise-minimized forward and reverse traces and review variant peak height percentages. As needed, double-click a variant in the top trace to edit a variant or add a comment. See also “How the software handles multiple variants at the same position” on page 45.
3. Use the center slider bar to zoom on the baseline for all six traces.
4. Type your name in the Reviewer name field at the top right of the screen, then add a comment as needed.
5. Click  to accept or click  to reject the variant.
6. *(Optional)* Select **Actions** ▶ **Generate Report** or **Actions** ▶ **Export Variants**.
7. (Not shown in figure) Press the right or left arrow keys on the keyboard to advance to the next or previous review position in the selected amplicon.



## 5 Summary

Verify that all variants are reviewed.

See also “How the software handles specimens in which no variants are found” on page 46.

Summary			
Number of Test Specimens:	1	Total number of Variants:	22
Number of Amplicons:	1	Number of Reviewed Variants:	0
Number of Traces Analyzed:	4	Number of Accepted Variants:	0
Number of Unreviewed Variants:	22	Number of Rejected Variants:	0
Number of Partially Reviewed Variants:	0		

Traces with Analysis Errors/Warnings			
Trace Name	Specimen	Amplicon	Errors/Warnings



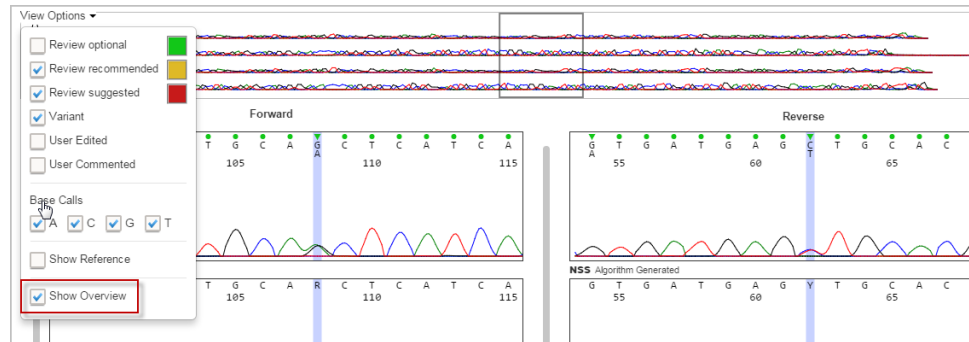
## 6 NGS Confirmation

If you specified a reference and .vcf file in the Reference screen, review Sanger variants that confirm Next Generation Sequencing results.

NGS Variants		Sanger Variants					
Chromosome	Position	Annotated Var...	Type	Ref	NGS...	San...	Review Status
7	55248995		Substitution	G	A	-	None
7	55249002		Insertion	C	CCAG...	-	None
7	55249005	rs121913465	Substitution	G	T	-	None
7	55249007	rs147149347	Substitution	G	T	-	None
7	55249010		Substitution	G	A	-	None
7	55249012		Insertion	C	CCGT	-	None

## Review electropherograms

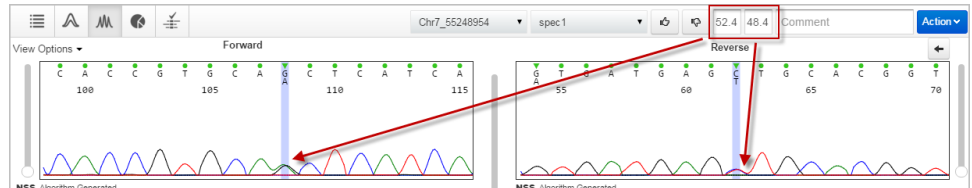
- (Optional) Select **View Options** ▶
  - **Show Overview** to display forward and reverse control and specimen traces. You can click a region in the overview traces to display the region in the electropherogram plots.
  - Other options to show the reference sequence and set the arrow key jump function for advancing to review positions (see “Set the arrow key jump function” on page 39).



- Type your name in the Reviewer name field at the top right of the screen, then add a comment as needed. You can alternatively add a comment if you edit a base, described later in this procedure.

- Select the amplicon and specimen of interest.

- Review variants in the top noise-minimized forward and reverse traces and review variant peak height percentages.



The variant peak height percentage can differ in the forward and reverse sequence for true variants. Extreme differences may indicate that the candidate variant is not a true variant.

Variant Percent:  $100 \times \text{Variant base peak height} / (\text{Primary} + \text{variant base peak heights})$

**IMPORTANT!** Sanger sequencing is not quantitative. Relative peak heights do not indicate the percent of the minor variant present in the DNA sequenced.

- As needed:
  - Scale the traces using the slider bar next to a trace or the center slider bar to scale all traces simultaneously.
  - Click-drag a trace or click in the Overview traces to display a different region.
  - Double-click a variant in the top noise-minimized trace to edit a base, then click **Enter**.

Edit Variant ⊞

A|

Comments

The review indicator color changes to gray for edited bases.

You can enter a comment in the Edit Variant dialog box (press **Enter** after typing the comment) or in the Comment field at the top right of the screen.

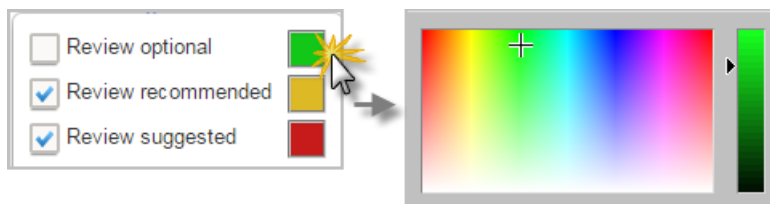
- Click to accept or click to reject the variant.
- Press the right or left arrow keys on the keyboard to advance to the next or previous review position in the selected amplicon.

## Results Review Indicator overview

The Results Review Indicator is an estimate of the risk of a false negative or false positive result at each base position. Color coding applies to variant and non-variant positions. Default colors are:

- Low risk, review optional
- Moderate risk, review recommended
- High risk, review suggested

To change the colors, select **View Options** in the Electropherogram screen, then click the color box for an indicator (the color change is not applied in the Variants screen).



**Note:** The Review Indicator color coding in Results is different from the quality indicator color coding in Traces.

A higher risk of a false negative or false positive result is present if:

- Quality Values assigned by the KB Basecaller are low
- Mixed bases identified by the KB Basecaller in one strand are not identified by the KB Basecaller in the opposite strand
- Signal-to-noise ratio for a possible variant peak is high in one strand but no complementary peak is observed in the opposite strand
- The primary base at a given position (or near a given position) is different in the control and specimen traces
- The score for a potential variant peak is near the threshold used to identify a variant (score and threshold are internal software values)

Review indicators are displayed in:

-  Variants screen


A...	Tes...	Chro...	P...	Va...	Edite...	Review sta...	Origin	An...	Review Indicator
E4B	15	E4B	161	C -> G	N	None	MVF		
E4B	7p5	E4B	161	C -> G	N	None	MVF		
E4B	50	E4B	161	C -> G	N	None	MVF		
E4B	7p5	E4B	322	G -> A	N	None	MVF		

-  Electropherogram screen



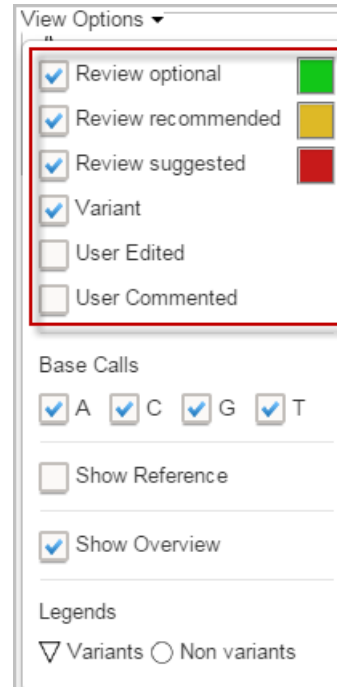
## Set the arrow key jump function

You can press right and left arrow keys on the keyboard to move the cursor to the next or previous position in a trace.

In the  Electropherogram screen:

Select **View Options**, then specify the next or previous function by selecting any of the following:




- Review indicator (optional, recommended, or suggested) to jump to a review indicator color (see “Results Review Indicator overview” on page 37)
- Variant to jump to any variant location
- User Edited or User Commented to jump to any location that has been edited or commented on

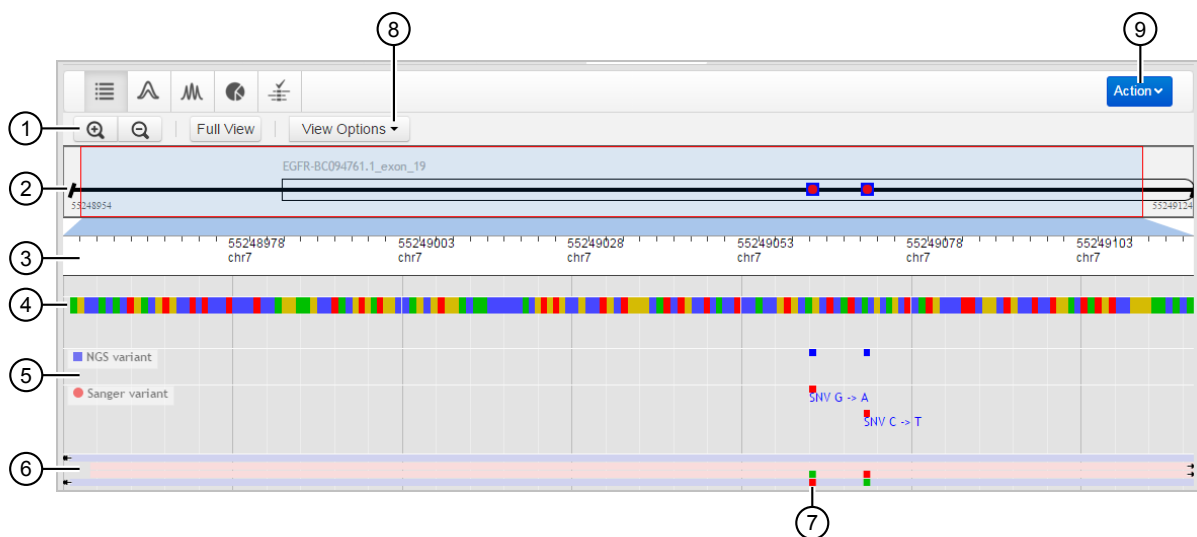


## Parts of the Results Overview screen

Click **Results** in the workflow bar to display the Results Overview screen.

Figure 3 Results screen

- ① Zoom/unzoom – Click   Full View to zoom and unzoom. As you zoom, the red display selector box moves to the region of the sequence shown in the zoom area.
- ② Reference overview bar – If you selected or created a reference in the Reference screen, shows an overview of exon and variant location in the reference sequence. See the figure below for more information.
- ③ Chromosome information
- ④ Control or reference sequence – Click anywhere in the sequence to zoom on that area of the sequence. Click  to show individual bases.
- ⑤ Variants [NGS [variant confirmation] or Sanger; SNV tags indicate the single nucleotide variant] – Click a variant to display variant details.
- ⑥ Sample sequences – Forward (pink) and reverse (blue). Top blue/pink = control; bottom pink/blue = sample.
- ⑦ Variants color-coded for the base
- ⑧ View Options – Select **Color by test specimen** to assign different colors to different specimens.
- ⑨ Action – Click to generate reports or export variants. PDF reports and .vcf/.csv export files are saved in the download location specified in the browser.





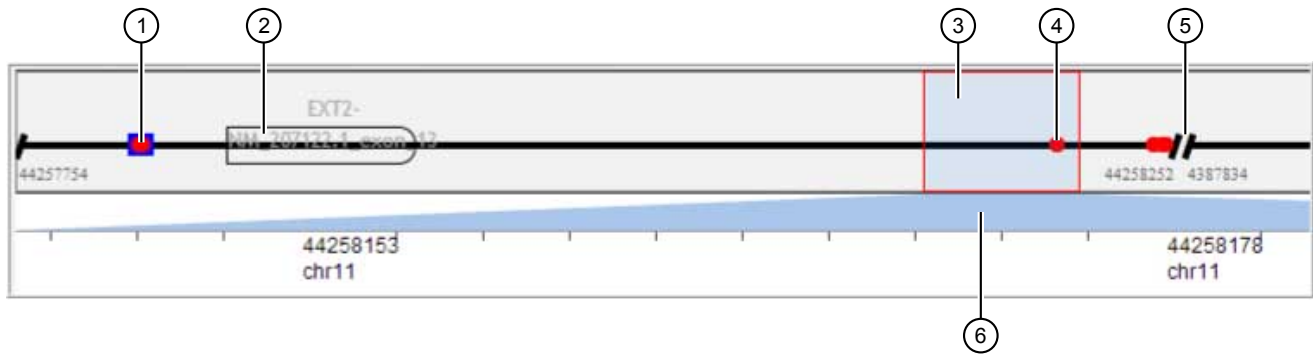


Figure 4 Reference overview bar

- ① Variant (Sanger [red circle] and NGS [blue square])
- ② Exon
- ③ Selector box – By default, the entire reference sequence is selected. Click drag over a region to zoom on a region. Click to the right or left of the box to move the box to the previous or next region. As you move the selector box, the blue shading below the box shows the chromosome region selected.
- ④ Variant (Sanger)
- ⑤ End/start of amplicon
- ⑥ Selected region indicator

## Parts of the Results Variants screen

Click **A** in the Results screen to display the Variants screen.

Variants													
Amplicon	Specimen	Chromosome	Position	Variant	Edited ...	Review status	Origin	Annotated Variant	Review Ind...	Forwar...	Revers...	Type	Variant Comments
Chr7_55248954	spec1	chr7	55249063	G -> A	N	None	Common	rs1050171		52.4	48.4	SNV	
Chr7_55248954	spec1	chr7	55249071	C -> T	N	None	Common			5.7	3.9	SNV	
Chr7_55248954		chr7	55248995	G -> A	N	None	NGS		N/A	N/A	N/A	Substitution	

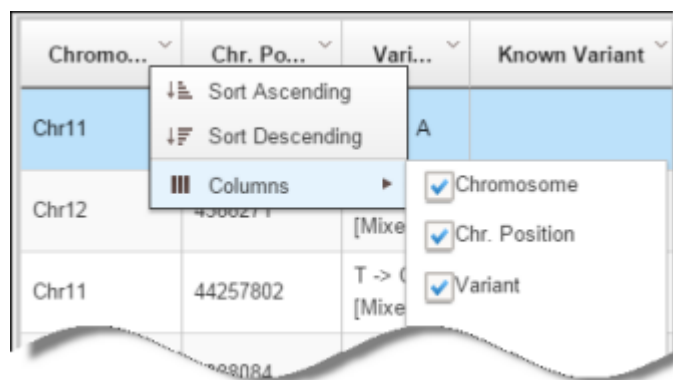
  

Non Variants													
Amplicon	Specimen	Chromosome	Position	Variant	Edited V...	Origin	Annotated Variant	Review Indic...	Forward ...	Reverse ...	Type	Variant Comments	


- ① Variants – Lists variants and review indicators (see “Results Review Indicator overview” on page 37). Double-click to display the electropherograms for a variant.
- ② Non-variants – Lists only non-variants with red review indicators. Double-click to display the electropherograms for a non-variant. If you edit a non-variant, it is moved to the variants table and assigned a Review status of On Hold.
- ③ Actions – Generate reports or export variants

To customize the table:

- Click table headers to sort columns.
- Click-drag table headers to move columns.
- Click the arrow next to any column header to select the columns to show or hide.



## Parts of the Results Electropherogram screen

Click  in the Results screen to display the Electropherogram screen.

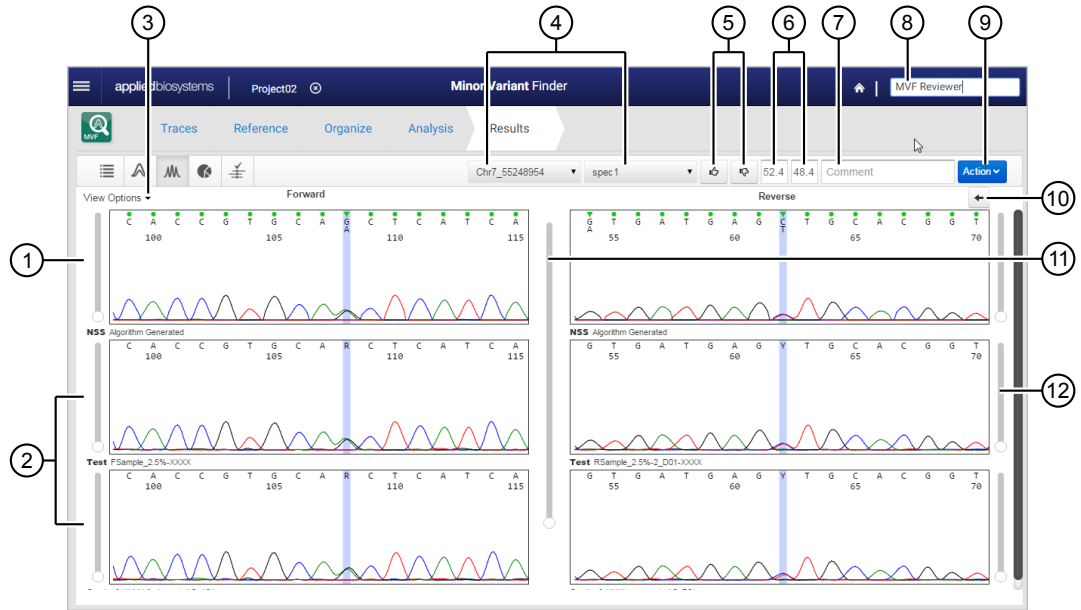


Figure 5 Parts of the Electropherogram screen

- ① Noise-minimized trace with variants (top letter=major peak; bottom letter=possible variant peak) and review indicators (see "Results Review Indicator overview" on page 37)
- ② Sample and control traces
- ③ View options to set arrow key function, display Reference trace, or display Overview trace
- ④ Amplicon and specimen selection lists
- ⑤ Accept and Reject the variant
- ⑥ Peak height percentages for major and variant peak  
Variant Percent:  $100 \times \frac{\text{Variant base peak height}}{\text{Primary} + \text{variant base peak heights}}$
- ⑦ Comment
- ⑧ Reviewer name (type to modify, up to 20 alphanumeric characters allowed)
- ⑨ Action – Generate report, export variants, and edit variants
- ⑩ Change to complement of reverse strand of the NSS trace
- ⑪ Scale all traces
- ⑫ Scale individual trace

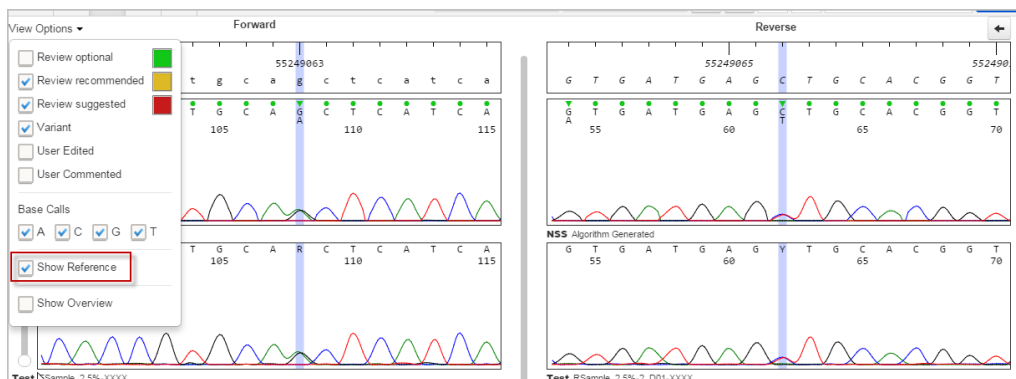


Figure 6 View Options>Show Reference

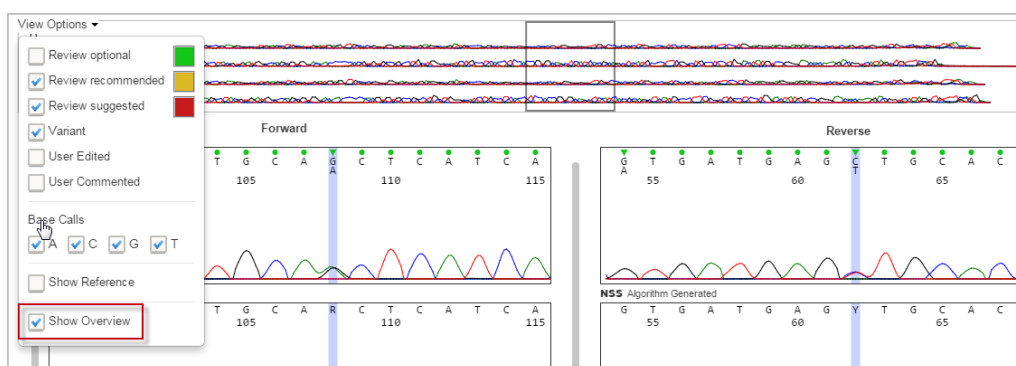


Figure 7 View Options>Show Overview

**Note:** By default, forward and reverse base selection is synchronized. However, if you zoom on a different region of the Overview trace, the reverse base is not automatically updated. Click a base in the forward sequence to update the base selection in the reverse sequence.

## Parts of the Results Summary screen

Click **Summary** in the Results screen to display specimen, amplicon, trace, and review information.

Summary			
Number of Test Specimens:	1	Total number of Variants:	22
Number of Amplicons:	1	Number of Reviewed Variants:	0
Number of Traces Analyzed:	4	Number of Accepted Variants:	0
Number of Unreviewed Variants:	22	Number of Rejected Variants:	0
Number of Partially Reviewed Variants:	0		


Traces with Analysis Errors/Warnings			
Trace Name	Specimen	Amplicon	Errors/Warnings

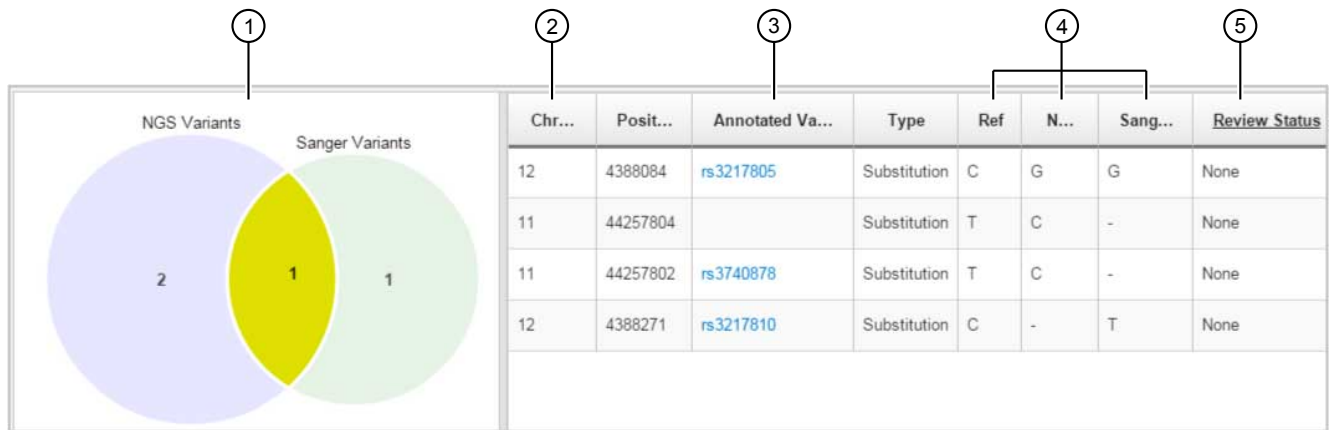
**Note:** If a specimen was successfully analyzed but no variants were identified, the specimen is listed under Traces with Analysis Errors\Warnings.

Review statuses:

- Reviewed = Accepted or Rejected
- Partially Reviewed = OnHold
- Unreviewed = No Review Status set in the Variants screen

## Parts of the Results NGS Confirmation screen

Click  in the Results screen to display the NGS Confirmation screen.




- ① Variant Venn diagram – Shows the number of NGS variants listed in the .vcf file selected in Setup, and the number of unreviewed or accepted Sanger variants (variants rejected in the Variants screen are not shown). The intersection of the two circles indicates the number of variants that are identified as both NGS and Sanger variants.
- ② Chromosome
- ③ Annotated Variant – Click to display NCBI information about the variant.
- ④ Reference base at the variant location and alternate base identified by the NGS and Sanger analyses.
- ⑤ Review Status – Status from the Variants screen.

## How the software handles multiple variants at the same position

If more than one variant is present at a given position, only one variant is reported in the base legend above the trace and in the variant peak height percentage (the variant with the largest secondary peak height). Review, then accept or reject the other variants in the Results Variants screen.

## How the software handles specimens in which no variants are found

If no variants are found in a specimen trace:

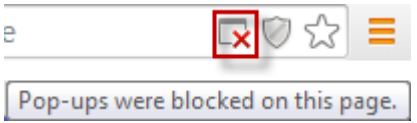
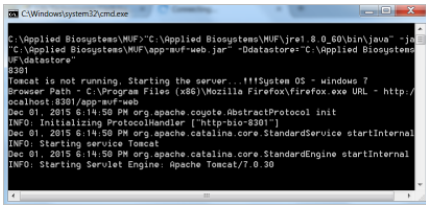
- The trace is listed in the  Summary screen in the Traces with Analysis Errors/Warnings list.
- No results are displayed for the trace in any of the other result screens.




# Troubleshooting

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## Troubleshooting

Observation	Possible cause	Action
Computers running Windows™ 10 and Microsoft™ Edge: Software does not launch.	The Google™ Chrome™ or Mozilla™ Firefox™ browser is required to start the software.	Uninstall the Minor Variant Finder Software, Install the Google™ Chrome™ or Mozilla™ Firefox™ browser, then reinstall the Minor Variant Finder Software.
No result report is displayed.	Browser pop-up blocker is preventing report from displaying.	Disable the pop-up blocker. <b>Note:</b> The procedure to disable a pop-up blocker varies based on browser. The example below shows the icon you click to disable a pop-up blocker in the Google™ Chrome™ browser. 
	Multiple reports are selected for downloading.	Select only one report for downloading at a time.
"Please try again" is displayed on a screen.	Tomcat™ software window was accidentally closed.	Restart the Minor Variant Finder Software.
Progress bar stops advancing, or software does not respond.		

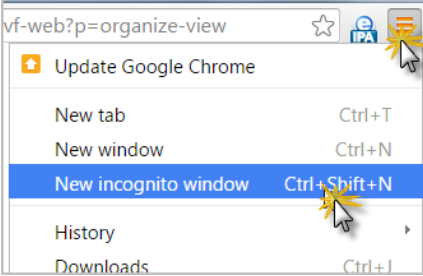
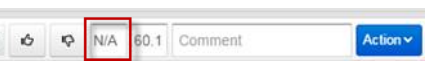
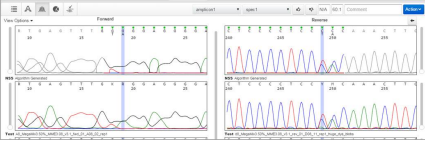


Observation	Possible cause	Action
The tab selected in the workflow bar does not match the screen displayed.	You clicked the browser Back button.	Do not click the browser Back button. Click a tab in the workflow bar to navigate to different screens.
Project screen: After exporting a project, the "Downloading" message is displayed and does not go away.	The browser is not refreshing.	Check the download directory to ensure that the .zip file for the project is downloaded, then refresh the browser window to remove the message.
A project is not imported and no message is displayed.	The .zip file for the project was unzipped then rezipped after export.	Repeat the project export, then import the untampered-with .zip file.
Reference screen: Import Assay IDs and Enter Chromosome Position options are grayed and not available for selection.	No internet connection available.	Specify regions of interest by entering a sequence.
		Connect to the internet before selecting the option.
Reference screen: "Uploading vcf file" message displayed, but file does not upload.	File name contains "%" character.	Refresh the browser, remove "%" character from the file name, then upload again.
Organize screen: Unable to drag control traces to Control column.	No amplicon name specified.	Add an amplicon name, then drag control traces.
Organize screen: Unable to drag sample traces to Amplicon column.	Amplicon column cannot contain sample traces.	Drag sample traces to the Grouped Traces column.
Organize screen: Control traces are grouped with the incorrect amplicon.	The Amplicon list is filtered.	Unfilter the Amplicon list before dragging the control trace to the Control box (click  , then delete the filter text).
Organize screen: When automatically organizing traces, a "No traces mapped to any amplicon" message is displayed.	<p>The requirements for automatic organization are not met.</p> <ul style="list-style-type: none"> <li>The name of the amplicon folder must match the part of the file name that you identify as amplicon.</li> <li>File names must contain "forward", "fwd", or "f" and "reverse", "rev", or "r" (case insensitive) characters preceded and followed by any of these delimiters -_\$.%.</li> <li>Two control traces can be added to an amplicon folder.</li> <li>Two specimen traces can be added to a specimen folder.</li> </ul>	Ensure requirements are met.





Observation	Possible cause	Action
<p>Analysis screen: Analysis failed message.</p>	<ul style="list-style-type: none"> <li>• The controls used are not appropriate for the specimen sequences in the project.</li> <li>• Incorrect test specimen traces used (for example, you used two forward test specimen traces instead of a forward and reverse).</li> <li>• The reference used is not appropriate for the control and specimen sequences in the project.</li> </ul>	<p>Specify the correct traces or reference.</p>
	<p>Traces with the same orientation were analyzed.</p>	<ol style="list-style-type: none"> <li>1. Review trace names in the Organize screen and ensure that one forward and one reverse trace are present in each specimen folder.</li> <li>2. Ungroup traces, then manually group one forward and one reverse trace in each specimen folder.</li> </ol>
	<p>The automatic organize function grouped Traces with the same orientation in a specimen folder.</p> <p>The part of the file name that represents the amplicon, specimen, or control name contains a delimiter character.</p>	<p>Do not include a delimiter character within the part of the file name that represents the amplicon, specimen, or control.</p>
	<p>Traces are not properly basecalled.</p>	<p>Rebasecall the .ab1 file in an application that uses KB Basecaller v1.4 or later.</p>
	<p>The overlap region of control and specimen traces is &lt;15 bp.</p>	<p>The software requires <math>\geq 15</math> bp overlap between control and specimen traces.</p>
	<p>Bases were inserted and/or deleted in the data before import into the Minor Variant Finder Software.</p>	<p>Data with edited bases cannot be analyzed with the Minor Variant Finder Software.</p>
	<p>Analysis screen: Analysis failed - No Sanger variants found for specimen message.</p>	<p>No variants are found in the specimen.</p>

Observation	Possible cause	Action
<p>Results Overview screen: "Not Found Error: Failed to execute insert before...."</p>	<p>A browser extension is conflicting with the display.</p>	<p>Open an incognito window, then paste the url from the window that displayed the error.</p> <p><b>Note:</b> The procedure to open an incognito window varies based on browser. The example below shows the Google™ Chrome™ browser.</p> 
<p>Results Overview screen: More than two variants at the same position are not shown.</p>	<p>The Results Overview screen shows one fewer variant than is listed in the Results Variants screen.</p>	<p>After reviewing variants in the Results Overview screen, return to the Results Variants screen to review variants that are not displayed in the Results Overview screen.</p>
<p>Results Overview screen: Variants are not shown in the reverse trace, traces are not aligned.</p>	<p>The orientation of the reference does not match the orientation of the forward control for the amplicon.</p>	<p>"Check the orientation of sequences in .ab1 files" on page 9.</p>
<p>Results Overview screen: Variants are not shown.</p>	<p>You did not specify a chromosome number and start position in the reference.</p>	<p>See "Create a new reference" on page 23.</p>
<p>Results Variant screen: The variant change is invalid (for example, T-&gt;T).</p>	<p>You edited a mixed base that is outside the analysis region two times. The software does not track changes outside the analysis region.</p> <p>For example, you edited a W base to an A (which is listed in the Variants screen as T-&gt;A), then edited the A to T (which is listed in the Variants screen as T-&gt;T).</p>	<p>You can re-analyze the project to correct the error. However, re-analyzing overwrites all base edits.</p>
<p>Results Electropherogram screen: Variant peak height percentage listed as N/A.</p> 	<p>Irregular peak shape in the corresponding sequence.</p> 	<p>Follow your laboratory protocol for evaluating variants when only one sequence strand is available for analysis.</p>

Observation	Possible cause	Action
Results Summary screen: Traces are listed with analysis errors or warnings.	<ul style="list-style-type: none"> <li>The controls used are not appropriate for the specimen sequences in the project.</li> <li>Incorrect test specimen traces used (for example, you used two forward test specimen traces instead of a forward and reverse).</li> <li>The reference used is not appropriate for the control and specimen sequences in the project.</li> </ul>	Specify the correct traces or reference.
	Bases were inserted and/or deleted in the data before import into the Minor Variant Finder Software.	Data with edited bases cannot be analyzed with the Minor Variant Finder Software.
	No variants were found, but analysis was successful.	No action.

## DNA Sequencing by Capillary Electrophoresis Guide

Go to [https://tools.lifetechnologies.com/content/sfs/manuals/cms\\_041003.pdf](https://tools.lifetechnologies.com/content/sfs/manuals/cms_041003.pdf) to access the Applied Biosystems™ DNA Sequencing by Capillary Electrophoresis Guide.

### Additional resources

- *Troubleshooting Sanger sequencing data* (Pub. no. MAN0014435)
- *Generating high-quality data using the BigDye™ Terminator v3.1 Cycle Sequencing Kit* (Pub. no. MAN0014628)
- *Generating high-quality data using the BigDye™ Direct Cycle Sequencing Kit* (Pub. no. MAN0014436)

# Documentation and support

## Related documents

Document	Publication number
<i>Minor Variant Finder Software Quick Reference</i>	MAN0014873

## Customer and technical support

Visit [thermofisher.com/support](http://thermofisher.com/support) for the latest in services and support, including:

- Worldwide contact telephone numbers
- Product support, including:
  - Product FAQs
  - Software, patches, and updates
- Order and web support
- Product documentation, including:
  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

**Note:** For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

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