Minor Variant Finder Software v1.0

Catalog Number A30835 Learn More

Pub. No. MAN0014873 **Rev.** A.0 Buy Now

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Product information

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.

This document contains workflows that illustrate how to use the software. For more information, refer to the *Minor Variant Finder Software User Guide* (Pub. no. MAN0014835).

The software is available for download at **www.thermofisher.com/mvf**.

Computer requirements

Component	Requirement
Computer	Windows™-compatible computer with 2 GB hard disk space and a minimum of 4 GB memory; 8 GB recommended
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
Screen resolution	1024 x 768 or higher, optimized for 1280 x 1024

Start the software

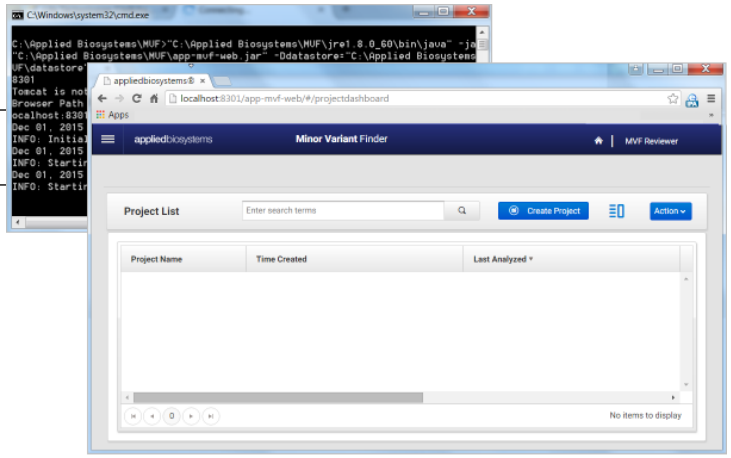
Select ▸ **All Programs** ▸ **Applied Biosystems** ▸ **MVF** ▸ **MVF** or double-click on the Windows™ desktop.

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.

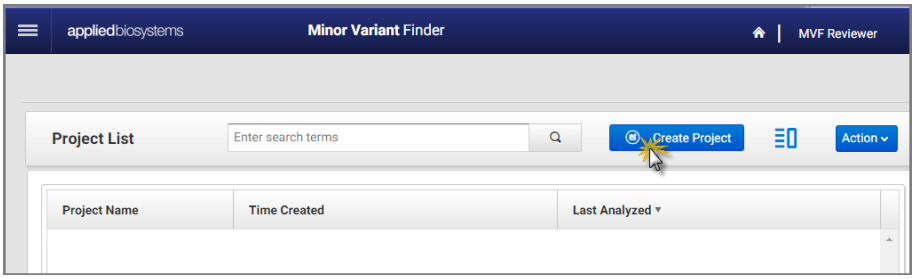
- A browser window that displays the Minor Variant Finder Software Project screen.
  By default, the software opens in Google™ Chrome™, but you can use any of the browsers listed in "Computer requirements" on page 1.



Project workflow

1 Project

Create a project



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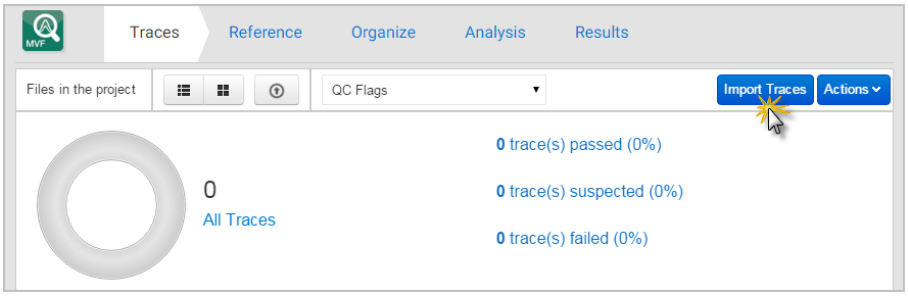
2 Traces

Import sample and control traces



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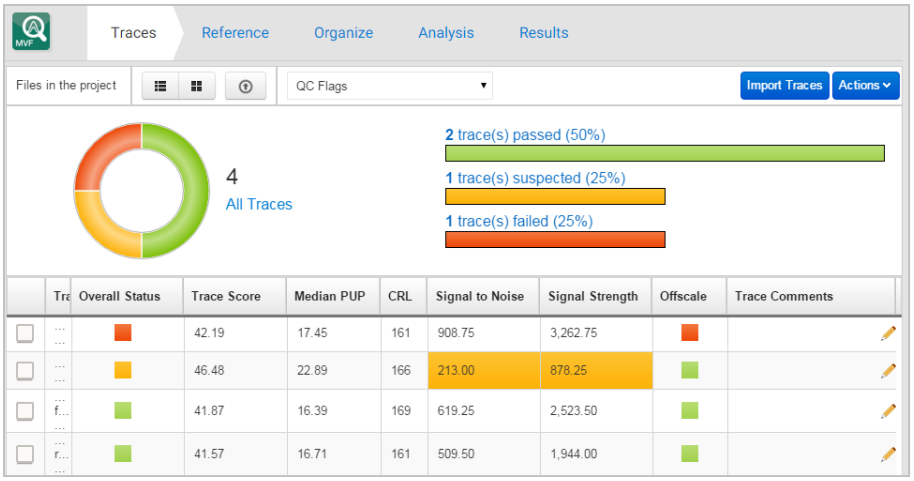
View trace quality



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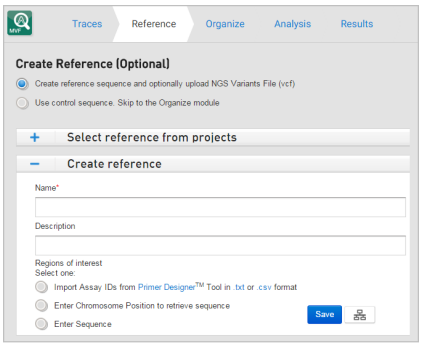
3 Reference

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:
   - 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



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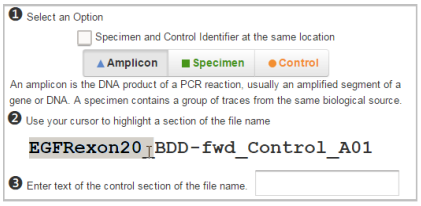
4 Organize

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.



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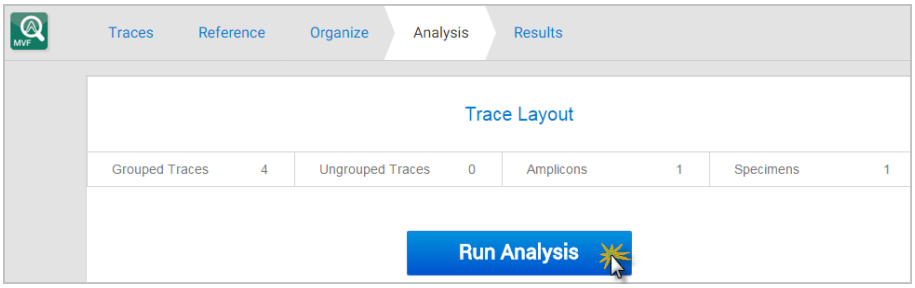
5 Analysis

Click **Run Analysis**.



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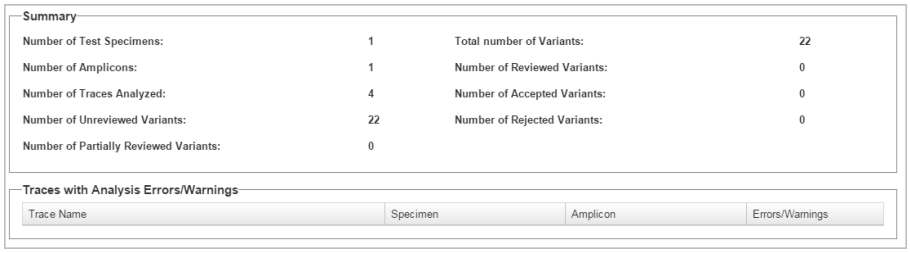
Results workflow

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.



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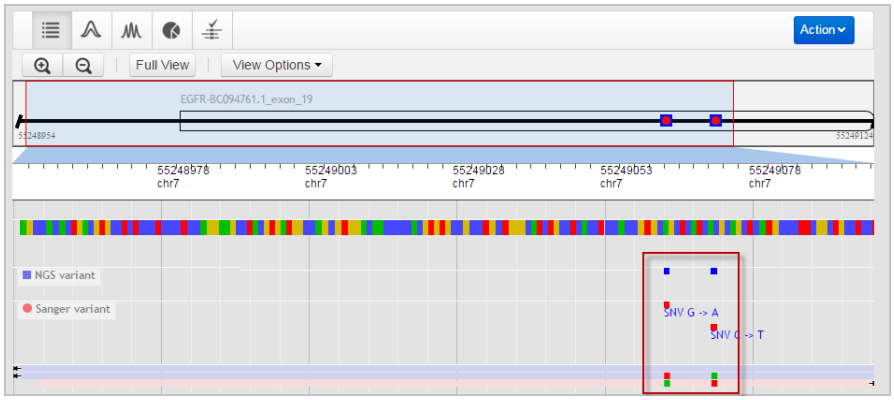
2 Overview

Identify the regions containing variants.



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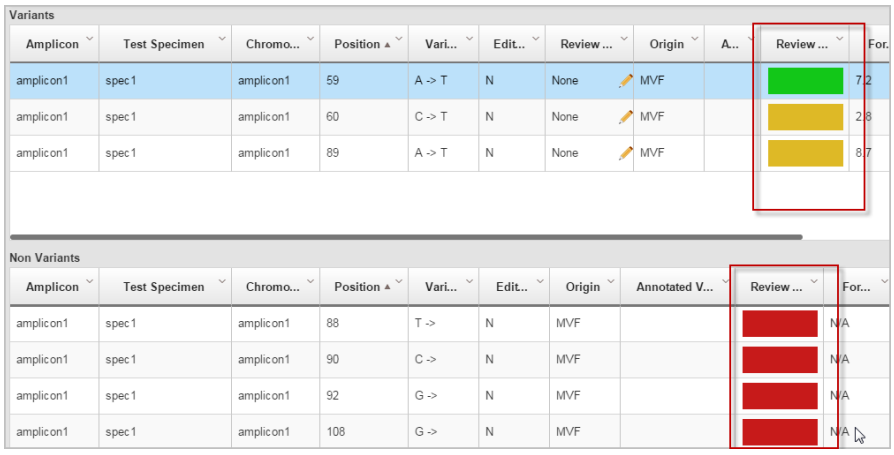
3 Variants

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).



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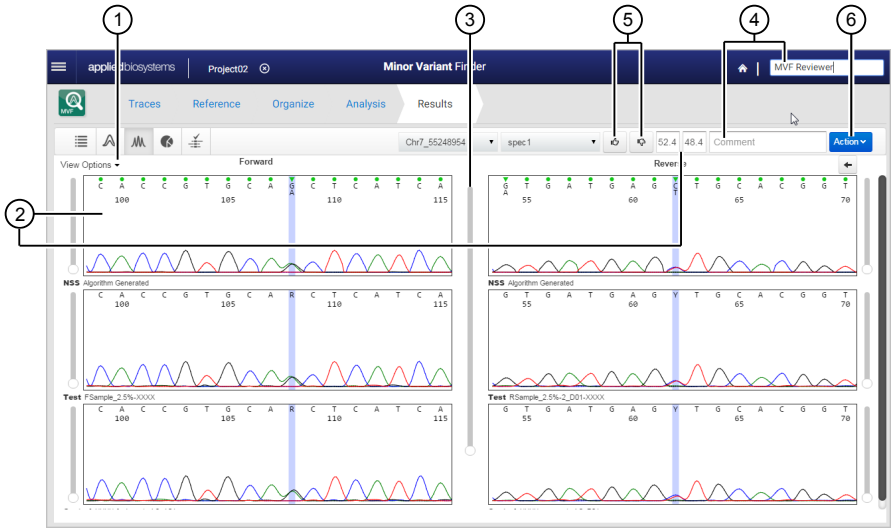
4 Electropherogram

1. *(Optional)* "Set the arrow key jump function" on page 6.
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.
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.



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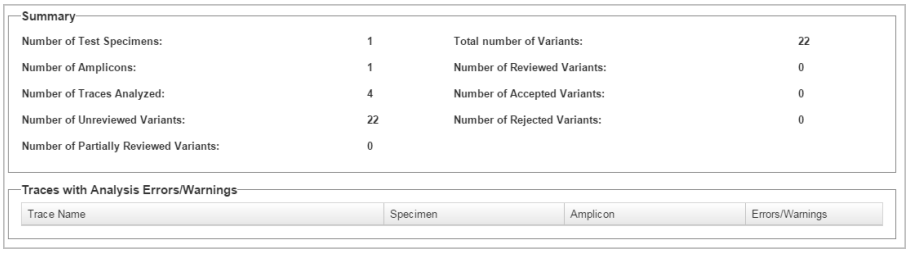
5 Summary

Verify that all variants are reviewed.



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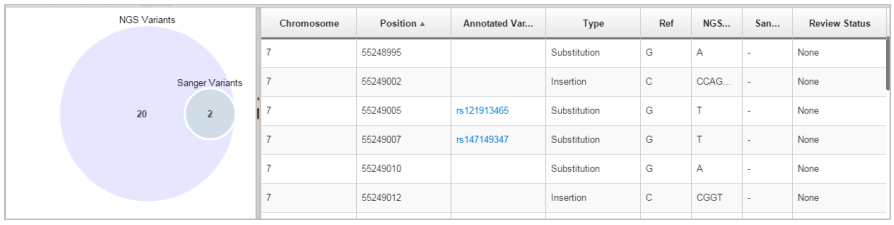
6 NGS Confirmation

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



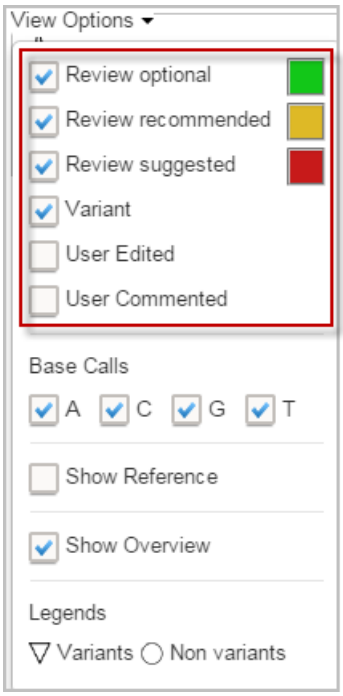
Set the arrow key jump function

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
- Variant to jump to any variant location
- User Edited or User Commented to jump to any location that has been edited or commented on



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**, 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**.
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.

   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

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.
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	To analyze an aplicon, the software requires: • 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 The software analyzes these four files simultaneously. The software does not support more than one set of controls per amplicon.
File names	Use the following naming convention for easy grouping of .ab1 files into specimens and amplicons before analysis: <*SpecimenOrControlName*>_<*AmpliconName (or Assay ID for Primer Designer™ Tool samples)*> _ <*Orientation*>_<*WellNo*>.ab1 Important requirements: • Include orientation characters "forward", "fwd", or "f" and "reverse", "rev", or "r" (case insensitive) in the file name to represent forward and reverse orientation. • Include one delimiter -_$.% before and after the orientation characters. The automatic organize function may not work without delimiter characters before and after orientation characters. • Do not include a delimiter character within the part of the file name that represents the amplicon, specimen, or control. Example of file names that will work properly with the organize function: • EGFR_exon_20_BDD-fwd_Control_A01.ab1 • EGFR_exon_20_BDD-fwd_Specimen5percent_E01.ab1 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: • EGFR_exon_20_BDD-fwd_*Specimen5%*_E01.ab1 • EGFR_exon_20_BDD-fwd_*Specimen7.5percent*_E01.ab1 • EGFR_*ex.on*_20_BDD-fwd_Specimen5percent_E01.ab1
Sequence orientation	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 6.
Data quality	For optimal results, the software requires high-quality data. See *Troubleshooting Sanger sequencing data* (Pub. no. MAN0014435) for additional information. 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: • *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)

The information in this guide is subject to change without notice.

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