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Revision History

Part #	Revision	Date	Description of Change
15040890	01	January 2017	Updated with descriptions of the following features introduced in VariantStudio v3.0: <ul style="list-style-type: none">• Annotation database Added feature to use a local copy of the annotation database.• Software interface Added descriptions of new columns in variants table. Added feature to show all variants in the variants table.• Reporting Noted that reports show shared variants.
15040890	E	June 2014	Release for VariantStudio v2.2, which has minor modifications to filtering, annotation, and file import and export.
15040890	D	December 2013	Updated to clarify VCF version requirements are 4.0, and later, and that only SNPs and indels are imported to VariantStudio.
15040890	C	November 2013	Updated to clarify functionality in VariantStudio v2.1: <ul style="list-style-type: none">• Importing Added best practices for importing classifications.• Annotating Noted that all genomic coordinates are on the positive strand. Noted that VariantStudio assumes that custom annotations are expressed on the positive strand.• Reporting Noted which transcripts are exported for variants that overlap multiple genes.

Part #	Revision	Date	Description of Change
15040890	B	October 2013	<p>Updated with descriptions of the following features introduced in VariantStudio v2.1:</p> <ul style="list-style-type: none"> • Software interface <p>Added description of gene table and no-call regions table.</p> <p>Added descriptions of new columns in variants table.</p> <p>Added description of settings for fixed columns and show/hide features.</p> <ul style="list-style-type: none"> • Importing <p>Added feature to import variants in exons with specified padding.</p> <p>Added description of gVCF files for import.</p> <p>Added feature to import variants from another sample to the current sample.</p> <ul style="list-style-type: none"> • Annotating <p>Added gene-level annotations.</p> <p>Added classification annotation and description of classification database.</p> <p>Expanded annotations from COSMIC database.</p> <p>Removed annotations previously derived from HGMD Professional.</p> <p>Added ClinVar, MedGen, GeneReviews, and SNOMED CT annotations sources.</p> <p>Updated annotation source versions for Ensembl, COSMIC, and NHLBI Variant Server.</p> <ul style="list-style-type: none"> • Filtering <p>Added family-based filter with descriptions of filtering logic.</p> <p>Added classification filter.</p> <p>Added advanced filter for complex Boolean expressions.</p> <ul style="list-style-type: none"> • Reporting <p>Added sample report feature with customizable report template.</p> <p>Added option to export all transcripts.</p> <p>For a complete list of features included in VariantStudio v2.1, see the software release notes provided with the software installer.</p>
15040890	A	May 2013	Initial release.

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Overview

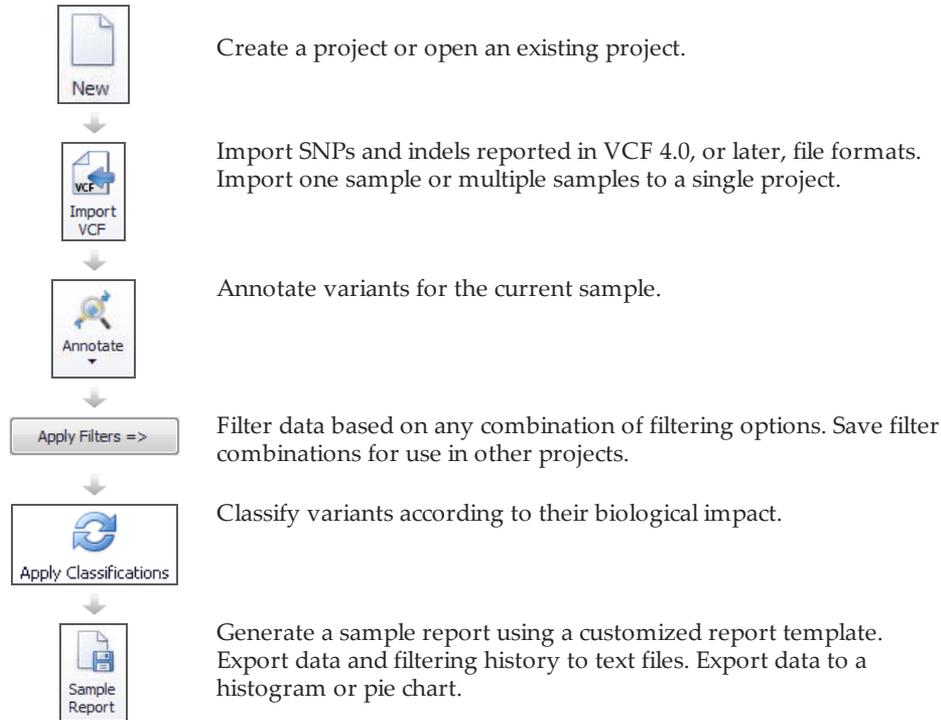
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Introduction

The VariantStudio™ software imports SNPs and indels reported in VCF v4.0, and later, file formats. After import, VariantStudio provides commands to annotate variants, filter results using filtering options, and export data to a report.

Figure 1 VariantStudio Workflow



System Requirements

VariantStudio software requires the following system specifications:

- ▶ 64-bit Windows OS (Windows 7, or later)
- ▶ 2 GB RAM minimum; 4 GB RAM recommended
- ▶ 20 GB hard drive space for installation

Install VariantStudio

- 1 Navigate to the VariantStudio support page, and then select **Downloads**.
- 2 Select **VariantStudio Software v3.0**.
- 3 Unzip the package, then double-click the software installer (*.msi) file.
- 4 Click **Next**.
The installation starts, and the progress screen opens. When the installation is finished, a desktop shortcut is created, and the setup complete screen opens.
- 5 Click **Finish**.



NOTE

Installation of VariantStudio v3.0 does not overwrite the version previously installed on your computer, allowing side-by-side installation of different software versions.

Install Database

VariantStudio uses a locally installed database to perform variant annotation.

- 1 Navigate to the VariantStudio support page, and then select **Downloads**.
- 2 Select **BaseSpace Annotation Engine**.
- 3 Create a folder named BaseSpace Annotation Engine on your C: drive.
- 4 Extract the database files to C:\BaseSpace Annotation Engine.
- 5 If the annotation database is moved to a folder other than C:\BaseSpace Annotation Engine, update the configuration file with the new database path.
 - a Open VariantStudio.exe.config in C:\Illumina\VariantStudio 3.0.
 - b Change the Annotation_RootPath value to the new annotation database location.
 - c Save the file.

VCF Input Requirements

VariantStudio imports SNPs and indels reported in VCF v4.0, or later, file formats. If analysis software other than Illumina analysis software is used to generate data, the VCF file might not contain the required columns.

VCF Column	Required Value
CHROM	The chromosome number. Values are #, c#, or chr#, where # is the chromosome number, as in 1–22, or name, as in X or Y, or M for mitochondrial.
POS	The position of the variant. Values are numeric with the first base having position 1 (1-based).
ID	The ID is the rs number for the SNP as recorded in dbSNP.txt. A value must be present. If a dbSNP entry does not exist, a missing value marker '.' is an acceptable value. Although the ID column and valid values are required, the values are not imported. The software applies dbSNP annotations with the Annotate command.
REF	The reference allele.
ALT	The alternate allele.
QUAL	The quality score assigned by the variant caller. A value of '.' is acceptable, and is reported as a 0.
INFO	Recognized fields are VF (alt variant freq), DP (read depth), AD (allelic depth), SOMATIC, and '.' (none). <ul style="list-style-type: none"> • VF—Represented in the Alt Variant Freq column in the Variants table. • DP—Represented in the Read Depth column in the Variants table. • DPI—Represented in the Read Depth column in the Variants table for insertion and deletion events called by the Illumina Isaac Alignment and Variant Calling workflow. • AD—Represented in the Alt Read Depth and Allelic Depth columns in the Variants table. • SOMATIC—Represented in the Genotype column in the Variants table. This value applies only to somatic variants.

VCF Column	Required Value
FORMAT	<p>A list of fields that define values in the Sample column. Possible values are VF (alt variant frequency), DP (read depth), AD (allelic depth), GT (genotype), and '.' (none).</p> <ul style="list-style-type: none"> • VF—Represented in the Alt Variant Freq column in the Variants table. • DP—Represented in the Read Depth column in the Variants table. • DPI—Represented in the Read Depth column in the Variants table for insertion and deletion events called by the Illumina Isaac Alignment and Variant Calling workflow. • AD—Represented in the Alt Read Depth and Allelic Depth columns in the Variants table. <p>Genotype Values: (Not present in somatic VCF files.)</p> <ul style="list-style-type: none"> • Acceptable GT values are 0/0, 0/1, and 1/1. Nonnumeric GT values, or './.' as in a no-call, are not imported. • Hemizygous alt GT values, '1', are accepted. Hemizygous reference calls, '0', are not imported. • If FORMAT and Sample are not empty, then a GT value is required. • IF FORMAT and Sample are empty, the software assumes that GT is heterozygous, 0/1.

Genome VCF Files

Importing genome VCF (gVCF) files is supported as of VariantStudio v2.1 for targeted enrichment data.

Using gVCF is not recommended for whole genomes without preprocessing with gVCF tools. Alternatively, you can load only exonic regions, or only regions from a gene list or BED file without the need for preprocessing. For more information, see *VCF Import Options* on page 21.

Viewing and Managing Data

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VariantStudio Software Interface

VariantStudio opens with a Start menu on the left panel.

Open or create a project to view the VariantStudio interface.

- ▶ Click **New Project**. The VariantStudio interface opens to a blank project.
- ▶ Click **Open Project** and browse to an existing project. Project names use the *.vbp file extension.



NOTE

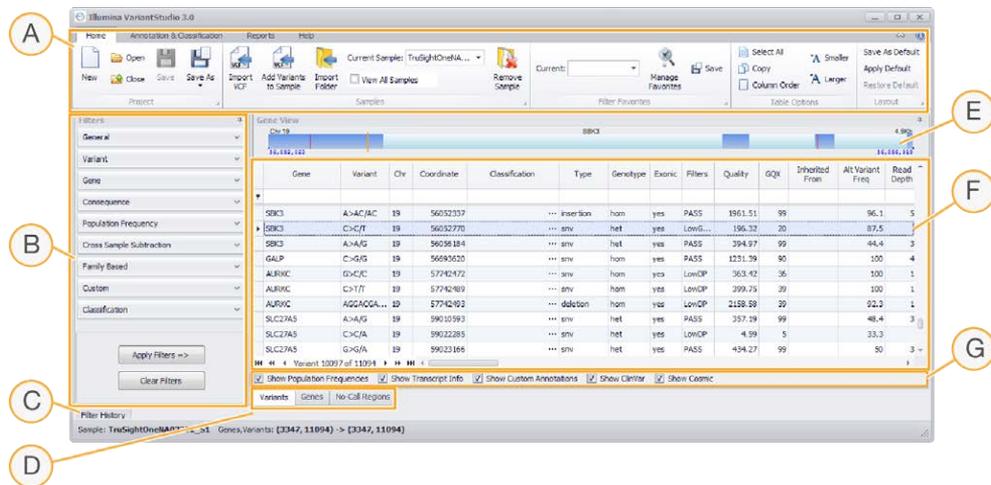
Projects created in VariantStudio v2.2 or earlier use a different database and are not compatible with VariantStudio v3.0. To use data from older projects, reimport the VCF files into a new VariantStudio v3.0 project.

- ▶ If recent projects are listed, click a project name from the list.

Interface Commands

The VariantStudio interface is an interactive view of genes and variants in a selected sample. Use the interface commands to import VCF files, sort data, apply filters, and export data to a report.

Figure 2 VariantStudio Interface



- A Menu and commands**—Contains commands for managing the project, annotating variants, and reporting results. Commands are organized in 4 tabs: Home, Annotation and Classification, Reports, and Help.
- B Filters pane**—Provides options for filtering data using any combination of filters.
- C Filter history**—Opens the history panel that shows all filters applied to the project.
- D Table tabs**—Navigates between the Variants table, Genes table, and No-Call Regions table.
- E Gene view**—Shows a graphical representation of the selected gene.
- F Table data**—Displays data in the Variants table, Genes table, or No-Call Regions table. Use the table tabs to select a table.
- G Show/hide columns**—Shows or hides groups of columns in the Variants table, Genes table, or No-Call Regions table.

Menus and Commands

VariantStudio commands are arranged in the following tabs:

- ▶ **Home tab**—Contains commands for saving projects, importing data, managing favorite filters, selecting samples, and changing layout options. For more information, see the following sections:
 - ▶ *Manage Samples* on page 21
 - ▶ *Modify Table Views* on page 22
 - ▶ *Create Favorite Filters* on page 48
- ▶ **Annotation and Classification**—Contains commands to annotate variants and apply classifications. For more information, see *Annotate Variants* on page 26.
- ▶ **Reports tab**—Contains commands for exporting results to reports. For more information, see *Generate Reports* on page 52.
- ▶ **Help tab**—Contains information about the software version and a link to the user guide. An internet connection is required to access or download the user guide.

Filters Pane

The Filters pane provides various filtering options to narrow results to your area of interest. Filters are applied to the current sample only, not to all samples that are imported into the project.

- ▶ Combine any number of filtering options from the filter categories and click **Apply Filters**.
- ▶ To clear filters, click **Clear Filters**.

For more information, see *Apply Filters* on page 36.

Filter History

The Filter History pane shows filters that have been applied to the samples in this project. Filters can be a single filter, a combination of filters, or a saved filter from the favorite filter list.

Figure 3 Filter History Pane

	Num Genes	Num Variants	Filter Name	Filter	
1	447	1969	Untitled	[]	Clear History
2	260	665	Untitled	[AlleleFreq < 17]	View Apply
3	61	91	Untitled	[AlleleFreq < 17 AND [Splice OR Inframe_Insertion OR Stop_Lost OR Inframe_Deletion OR Initiator_Codon OR Stop_Gained OR Frameshift OR Missense]]	View Apply

Column Heading	Description
Num Genes	The number of genes in the table with the filters applied.
Num Variants	The number of variants in the table with the filters applied.
Filter Name	The name of the filter applied. The filter name appears only if the filter was saved as a favorite. Otherwise, the filter name is Untitled.
Filter	The configuration of the applied filter or combination of filters.

The Filter History pane includes the following options.

- ▶ **Clear History**—Clears entries in the filter history pane.
- ▶ **View**—Shows a block diagram illustration of the filter.

- ▶ **Apply**—Applies the filter to the variants table.

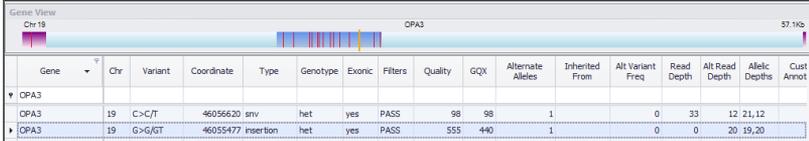
You can export information from the Filter History pane to a comma-separated values (CSV) file. For more information, see *Generating Reports* on page 51.

Gene View

The Gene View shows a graphical representation of the gene with the following indicators:

- ▶ Exons are indicated in dark blue.
- ▶ Variants are indicated with a red line.
- ▶ The selected variant is indicated with an orange line.
- ▶ The selected transcript is indicated in purple.
- ▶ No-call regions are indicated in gray.

Figure 4 Gene View



Gene	Chr	Variant	Coordinate	Type	Genotype	Exonic	Filters	Quality	GQX	Alternate Alleles	Inherited From	Alt Variant Freq	Read Depth	Alt Read Depth	Allelic Depths	Cust Annot
OPA3	19	C>C/T	46055620	snv	het	yes	PASS	98	98	1		0	33	12	21,12	
OPA3	19	G>G/GT	46055477	insertion	het	yes	PASS	555	440	1		0	0	20	19,20	

The Gene View is interactive. Using your mouse, hover over the view to see the coordinate. Click and drag your mouse to slide the view from end to end. Use the scrolling feature on your mouse to zoom in and zoom out.

Data in VariantStudio Tables

Imported and annotated information for the visible sample is arranged in 3 tables on the VariantStudio interface: Variants table, Genes table, and No-Call Regions table. Use the tabs below the table area to navigate between tables.

Figure 5 Navigation Tabs for Variants Table, Genes Table, and No-Call Regions Table

Gene	Variant	Chr	Coordinate	Type	Genotype	Exonic	Filters	Quality	GQX	Alternate Alleles
OR4F5	A>G/G	1	69270	snv	hom	yes	LowG...	123	24	2
OR4F5	A>G/G	1	69270	snv	hom	yes	LowG...	123	24	2
OR4F5	A>G/G	1	69511	snv	hom	yes	PASS	226	42	2
OR4F5	A>G/G	1	69511	snv	hom	yes	PASS	226	42	2
OR4F5	T>C/C	1	69897	snv	hom	yes	LowG...	144	18	2
OR4F5	T>C/C	1	69897	snv	hom	yes	LowG...	144	18	2
SAMD11	T>C/C	1	877831	snv	hom	yes	PASS	573	126	2
SAMD11	T>C/C	1	877831	snv	hom	yes	PASS	573	126	2
SAMD11,NOC2L	G>A/A	1	879676	snv	hom	yes	PASS	706	163	2
SAMD11,NOC2L	G>A/A	1	879676	snv	hom	yes	PASS	706	163	2

Variant 7 of 98192

Show Population Frequencies
 Show Transcript Info
 Show Custom Annotations
 Show ClinVar
 Show Cosmic

Variants Table

The Variants table lists the genes that overlap variants identified in the selected sample. Each row of the table contains the gene and reported variant. Genes that include multiple variants are listed multiple times in the table, 1 time for each variant.

The following information is provided in the Variants table. Some columns are hidden by default. If a column described in the following is not visible in your instance of VariantStudio, click **Column Order** from the Table Options menu to change the display setting.

Column Heading	Description
Sample	The name of the sample. This column is displayed only when View All Samples is selected.
Gene	The name of the gene.
Variant	Lists the reference allele and the diploid genotype call for the sample as Reference > AlleleA/AlleleB. AlleleA and AlleleB are explicitly defined from the REF, ALT, and GT fields of the VCF file. For example, at a heterozygous position noted as GT=0/1 is represented as REF > REF/ALT, and a homozygous nonreference position noted as GT=1/1 is represented as REF > ALT/ALT.
Chr	The chromosome number in which the gene occurs.
Coordinate	The genomic location of the variant (1-based).

Column Heading	Description
Classification	The classification assigned to the variant. This field is populated for variants that match criteria specified in the classification database.
Type	The type of variant, which is either a single nucleotide variant (SNV), insertion, deletion, or ref (for reference call).
Genotype	The genotype, which is either heterozygous (het), homozygous (hom) or somatic (som).
Exonic	A variant found within a coding region, ± 20 bp on both sides of the coding region.
Filters	The status of the variant call quality as annotated in the VCF file. PASS indicates that all filters were passed; otherwise the variant call filter is listed. The filter listed and threshold for passing filter depends on the method used to generate the VCF file.
Quality	The numeric value of variant call quality as written to the QUAL column of the VCF file. Determination of variant quality depends on the variant caller.
GQX	The conservative measure of genotype quality derived from the minimum of the GQ and QUAL values listed in the VCF file. This field is not populated for somatic VCF files. For more information, see <i>Somatic VCF Fields Reported in the Variants Table</i> on page 16.
Inherited From	The inherited source of the variant. Possible values are father, mother, both, indeterminate, or ambiguous. If the variant is heterozygous in the father, mother, and child, a variant is listed as ambiguous. If the variant is homozygous in the child and heterozygous in both parents, a variant is listed as both. If the inheritance of the variant cannot be determined from the other VCF files, the variant is listed as indeterminate. Entries in this column are meaningful only when the family-based filter is applied.
Alt Variant Freq	The frequency of the Alt Allele.
Read Depth	The total number of reads passing quality filters at this position.
Alt Read Depth	The number of reads called at this position.
Allelic Depths	The number of reads called for the Ref Allele and the Alt Allele.
Custom Annotation	Annotations according to values provided in the Annotation column of an optional custom annotation file.
Custom Gene Annotation	Annotations according to values provided in the Annotation column of an optional custom gene annotation file.
Custom Gene Annotation 2	Annotations according to values provided in the Gene Annotation 2 column of an optional custom annotation file.

Column Heading	Description
Num Transcripts	The number of transcripts reported in the annotation, which includes overlapping transcripts and upstream and downstream transcripts within 5 kb of the variant.
Transcript	The name of the transcript, usually a database identifier from RefSeq or Ensembl.
Consequence	Consequence of the variant, described in Sequence Ontology standardized vocabulary.
cDNA Position	Position of the variant in cDNA.
CDS Position	Position of the variant in the coding region.
Protein Position	Position of the amino acid in the protein
Amino Acids	Amino acid or amino acid change. If the variant is synonymous, then there is no change and 1 amino acid is listed.
Codons	Specific codon noted with and without the variant, highlighted in uppercase.
Transcript HGNC	The transcript name, expressed as official HGNC nomenclature.
Canonical	Indicates whether the transcript is the canonical transcript.
SIFT	SIFT score.
PolyPhen	PolyPhen score.
ENSP	Protein ID (Ensembl ID).
HGVSc	Human Genome Variation Society (HGVS) notation in the cDNA.
HGVSp	Human Genome Variation Society (HGVS) notation in the protein.
dbSNP ID	The rsID entry in dbSNP.
Ancestral Allele	The inferred allele ancestral to the chimpanzee/human lineage. For more information, see www.1000genomes.org/faq/where-does-ancestral-allele-information-your-variants-come .
Allele Freq	The allele frequency from all populations of 1000 genomes data.
Allele Freq Global Minor	Global minor allele frequency (GMAF); technically, the frequency of the second most frequent allele. For more information, see www.ncbi.nlm.nih.gov/projects/SNP/docs/rs_attributes.html#gmaf .
Global Minor Allele	The specific allele with the reported GMAF.
Allele Freq Amr	The allele frequency from 1000 Genomes (Ad Mixed American population).
Allele Freq Eas	The allele frequency from 1000 Genomes (East Asian population).
Allele Freq Sas	The allele frequency from 1000 Genomes (South Asian population).

Column Heading	Description
Allele Freq Af	The allele frequency from 1000 Genomes (African population).
Allele Freq Eur	The allele frequency from 1000 Genomes (European population).
EVS Freq All	The allele frequency from Exome Variant Server (EVS), all populations.
EVS Coverage	The average depth of coverage for SNVs that were called at this position from the Exome Variant Server (EVS).
EVS Samples	The number of samples that were called at this position from the Exome Variant Server (EVS).
ExAC Freq All	The allele frequency of the variant in the Exome Aggregation Consortium (ExAC) database, all populations.
ExAC Freq Afr	The allele frequency of the variant in the ExAC database, African/African American population.
ExAC Freq Amr	The allele frequency of the variant in the ExAC database, Latino population.
ExAC Freq Eas	The allele frequency of the variant in the ExAC database, East Asian population.
ExAC Freq Sas	The allele frequency of the variant in the ExAC database, South Asian population.
ExAC Freq Fin	The allele frequency of the variant in the ExAC database, Finnish population.
ExAC Freq Nfe	The allele frequency of the variant in the ExAC database, non-Finnish population.
ExAC Freq Other	The allele frequency of the variant in the ExAC database, other populations.
ExAC Coverage	Approximate read depth (reads with MQ=255, or with bad mates, are filtered) in the ExAC database.
ExAC Samples	Total number of alleles in called genotypes in the ExAC database.
Conserved Sequence	Denotes if the variant is an identical or similar sequence that occurs between species and maintained between species throughout evolution.
COSMIC ID	The numeric identifier for the variant in Catalogue of Somatic Mutations in Cancer (COSMIC) database, if the genomic position of the variant overlaps a variant listed in COSMIC. The COSMIC ID links to the COSMIC page associated with the identifier.
COSMIC Allele	The allele as reported in the COSMIC database.
COSMIC Gene	The gene name as reported in the COSMIC database.
COSMIC Primary Site	The primary tissue type associated with the allele as reported in the COSMIC database.

Column Heading	Description
COSMIC Histology	The tissue type associated with the allele as reported in the COSMIC database.
ClinVar Accession	The alpha-numeric ID assigned to the allele in the ClinVar database and link to the associated page of the ClinVar database.
ClinVar Alleles	The name of the allele as reported in the ClinVar database.
ClinVar Allele Type	The type of allele, either single nucleotide variant (SNV), insertion, deletion, or duplication as reported in the ClinVar database.
ClinVar Significance	The clinical significance or classification assigned to the allele as reported in the ClinVar database.
ClinVar RS	The numeric rsID assigned to the allele in the ClinVar database.
ClinVar Disease Name	The disease associated with the allele as reported in the ClinVar database.
ClinVar MedGen	The alpha-numeric identifier of the disease as reported by MedGen and link to the associated page of the MedGen database.
ClinVar OMIM	The numeric identifier for the disease as reported by Online Mendelian Inheritance in Man (OMIM) and link to the associated page of the OMIM database.
ClinVar Orphanet	The numeric identifier of the disease as reported by Orphanet and link to the associated page in the Orphanet database.
ClinVar Gene Reviews	The alpha-numeric identifier of the disease as reported by Gene Reviews and link to the associated page in the Gene Review database.
ClinVar SnoMedCt ID	The numeric identifier of the disease and associated clinical terms as reported by SnoMedCt.
Regulatory Feature	A link to regulatory information in Ensembl for that genomic region.
Alternate Alleles	The number of nucleotide bases called for Allele A and Allele B that differ from the RefAllele.
Google Scholar	Link to the Google Scholar search page for the selected variant, cDNA, and amino acid. The page opens with an autopopulated search field. Click search to continue. The search is transcript-dependent. If a rs number is present, the number is included in the search.
PubMed	Link to PubMed search page for the selected variant, cDNA, and amino acid. The page opens with an autopopulated search field. Click search to continue. The search is transcript-dependent. If a rs number is present, the number is included in the search.
UCSC Browser	Link to UCSC Browser search page for the selected chromosome and position.
Alternate Allele	The alternate allele for this variant.

Column Heading	Description
Exon	The exon number in which the variant is present.
Intron	The intron number in which the variant is present.

VCF Fields Reported in the Variants Table

Several columns of the Variants table are populated from columns or fields in the VCF file, as described in the following table.

Variants Table Column Heading	VCF File Column or Field Description
Allelic Depth	Based on values listed for AD in INFO or FORMAT/[Sample Name].
Alt Read Depth	Based on the second value listed for AD in INFO or FORMAT/[Sample Name].
Alt Variant Freq	Based on values listed for VF in INFO or FORMAT/[Sample Name].
Chr	Based on values in the CHROM column.
Coordinate	Based on values in the POS column.
Exonic	Based on values in the CHROM and POS columns, and calculated from a list of exonic regions.
Filters	Based on values in the FILTER column.
Gene	Based on values in the CHROM and POS columns, and calculated using a list of gene coordinates.
Genotype	Based on values listed for GT in FORMAT/[Sample Name].
GQX	Based on values listed for GQX in FORMAT/[Sample Name].
Quality	Based on values in the QUAL column.
Read Depth	Based on values listed for DP in FORMAT/[Sample Name], DPI in FORMAT/[Sample Name] for insertion and deletion events called by the Illumina Isaac Alignment and Variant Calling workflow.
Type	Based on the number of bases in the REF and ALT columns.
Variant	Based on values in the REF and ALT columns. <ul style="list-style-type: none"> At a heterozygous position, the value is REF > REF/ALT. At a homozygous position, the value is REF > ALT/ALT.



NOTE

Some fields reported in the Variants table differ for somatic VCF files. For more information, see *Somatic VCF Fields Reported in the Variants Table* on page 16.

Somatic VCF Fields Reported in the Variants Table

Information reported in VariantStudio for VCF files generated by the Illumina cancer analysis pipeline differs from what is reported for other VCF files.

For these files, there is no genotype (GT) or genotype score (GQX). Instead, allelic depths are listed.

Each VCF includes a cancer sample and a reference sample. All reported values are specific to the cancer sample.

The following table lists the VCF fields that are unique to somatic VCF files.

Variants Table Column Heading	VCF File Column or Field Description
Allelic Depth	<p>Based on values in the FORMAT column. Allelic Depth is calculated differently for SNVs and indels:</p> <ul style="list-style-type: none"> • For SNVs—Based on 4 values listed as AU:CU:GU:TU in the FORMAT column. These values are listed as 2 numbers each, separated by a comma, and represent each possible allele in the cancer sample. The Allelic depth column is populated with the full set of numbers, 0,0:0,0:10,10:3,4. • For indels—2 values listed as TAR:TIR in the FORMAT column represent the Ref Allele and Alt Allele, respectively. Only the first number in each value is used. In the example 0,0:12,12, the Ref Allele is 0 and the Alt Allele is 12. Allelic Depth is listed as 0,12.
Alt Read Depth	<p>Based on values in the FORMAT column. Alt Read Depth is calculated differently for SNVs and indels:</p> <ul style="list-style-type: none"> • For SNVs—Based on the first value from the appropriate Allelic Depth (AU:CU:GU:TU). In the example 0,0:0,0:10,10:3,4, the values are 10,10 for GU and 3,4 for TU. If the Ref Allele is G and the Alt Allele is T, the Alt Read Depth is 3. • For indels—Based on the first value from the appropriate Allelic Depth (TAR:TIR). In the example 0,0:12,12, the Ref Allele is 0 and the Alt Allele is 12. Alt Read Depth is listed as 12.
Alt Variant Freq	<p>For somatic VCF files, allele frequency is calculated from values in the VCF file before data are reported in the Variants table.</p> <ul style="list-style-type: none"> • For SNVs—Using only the first values for AU:CU:GU:TU, allele frequency is calculated as (alt allelic depth/(alt allelic depth + ref allelic depth))*100. In the example 0,0:0,0:10,10:3,4, Alt Variant Freq is 23.08% by calculating $(3/(3+10))*100$. • For indels—Using only the first values for TAR and TIR, allele frequency is calculated as $(TIR/(TIR+TAR))*100$. In the example 0,0:12,12, Alt Variant Freq is 100% by calculating $(12/(12+0))*100$.
Genotype	<p>Based on values listed in the INFO column. If SOMATIC is listed in the INFO column, the genotype is listed as somatic (som) in the Variants table.</p>
Quality	<p>Quality is based on different values for SNVs and indels.</p> <ul style="list-style-type: none"> • For SNVs—Quality is based on the QSS_NT field in the INFO column. This score represents the probability that the SNV exists and is somatic. • For indels—Quality is based on the QSI_NT field in the INFO column. This score represents the probability that the indel exists and is somatic.
Read Depth	<p>For SNVs and indels, Read Depth is extracted from values listed for DP in the FORMAT column of the cancer sample.</p>

Genes Table

The Genes table lists the genes that contain variants identified in the selected sample. Each row of the table contains the gene and number of variants reported, along with the following information reported for each gene.

Column Heading	Description															
Name	The name of the gene.															
Gene ID	The Entrez Gene ID for the gene and link to the associated entry in the NCBI database.															
Chr	The chromosome number in which the gene occurs.															
Start	The start coordinate of the gene (1-based).															
Stop	The end coordinate of the gene.															
Length	The length of the gene.															
Num Alleles	The number of alleles reported.															
Num Variants	The number of variants reported.															
Paternal Variants	The number of variants inherited from the father.															
Maternal Variants	The number of variants inherited from the mother.															
Ambiguous Variants	The number of ambiguous variants.															
Custom Gene Annotation	Gene annotations according to values provided in the Annotation column of an optional custom gene annotation file.															
Custom Gene Annotation 2	Annotations according to values provided in the Gene Annotation 2 column of an optional custom annotation file.															
PubMed	Link to PubMed search page for the selected gene. The page opens with an autopopulated search field. Click search to continue.															
GeneReviews	Link to GeneReviews website. Clicking the entry provides a drop-down list with links to GeneReviews.															
Disease	<p>Diseases associated with the gene. Click an entry to view a list of the associated diseases in a table.</p> <table border="1"> <thead> <tr> <th colspan="3">Familial hypercholesterolemia; Tangier disease; Familial hypoalphalipoproteinemia</th> </tr> <tr> <th>MedGen</th> <th>OMIM</th> <th>Disease</th> </tr> </thead> <tbody> <tr> <td>C0020445</td> <td>143890</td> <td>Familial hypercholesterolemia</td> </tr> <tr> <td>C0039292</td> <td>205400</td> <td>Tangier disease</td> </tr> <tr> <td>C1704429</td> <td>604091</td> <td>Familial hypoalphalipoproteinemia</td> </tr> </tbody> </table> <ul style="list-style-type: none"> • Use the sort and filter options to narrow the list. • Click a link in the table to view the MedGen and OMIM entries. 	Familial hypercholesterolemia; Tangier disease; Familial hypoalphalipoproteinemia			MedGen	OMIM	Disease	C0020445	143890	Familial hypercholesterolemia	C0039292	205400	Tangier disease	C1704429	604091	Familial hypoalphalipoproteinemia
Familial hypercholesterolemia; Tangier disease; Familial hypoalphalipoproteinemia																
MedGen	OMIM	Disease														
C0020445	143890	Familial hypercholesterolemia														
C0039292	205400	Tangier disease														
C1704429	604091	Familial hypoalphalipoproteinemia														
Description	The protein name associated with gene function.															

No-Call Regions Table

The No-Call Regions table shows regions where calls could not be confidently made due to a low read depth or failing a quality filter. The No-Call Regions table is populated directly from the nonvariant regions reported in the genome VCF (gVCF) file. Nonvariant regions are reported as 0/0 in the gVCF file. For more information, see sites.google.com/site/gvcftools/home/about-gvcf/gvcf-conventions

Each row of the table contains the gene and information reported for the gene. The following information is provided in the No-Call Regions table.

Column Heading	Description
Gene	The name of the gene located within the no-call region.
Chr	The chromosome number in which the no-call region occurs.
Start	The start chromosomal coordinate of the no-call region.
Stop	The end chromosomal coordinate of the no-call region.
Length	The length of the no-call region.
Depth	The read depth of the no-call region.
Quality	The numeric value of variant call quality as written to the QUAL column of the VCF file. Determination of variant quality depends on the variant caller.
Filter	The filter associated with the variant call quality as annotated in the VCF file.

Manage Projects

The Project menu includes commands to create, open, save, and name projects.

Command	Description
New	Creates a project.
Open	Opens a project.
Close	Closes the current project.
Save	Saves the current project.
Save As	Save As... —Saves a copy of the current project. Save As Reduced Project —Saves the filtered data of the current project as a new project. You cannot recover variants that were filtered out when saving at a reduced size.

Manage Samples

From the Home tab, use commands on the Samples menu to import variant call files in VCF file format and manage samples in the project.

Command	Description
Import VCF	Imports samples from a VCF file.
Add Variants to Sample	Imports SNPs and indels from another VCF file. This command imports data from the selected VCF file and adds it to the current sample. Important: There is no change to the sample name to denote that variant calls have been merged.
Import Folder	Imports all VCF files in a selected folder.
Current Sample	Shows the active sample name. To view data from a different sample, select the sample name from the drop-down list.
View All Samples	Shows all samples in the project.
Remove Sample	Removes the current sample from the project.

VCF Import Options

Option	Description
All variants	Imports all variants in the selected VCF files.
Variants in exons	Imports only variants found in exonic regions. Set the number of bases, or padding, to include on both sides of the exon.
Variants specified in a gene list	Imports variants from a gene list. The gene list file must be a text file with a *.txt file extension that lists one gene per line.
Variants in regions specified in a BED file	Imports variants from a BED file.
Load hom-ref positions	This setting applies to gVCF files. <ul style="list-style-type: none"> Select the checkbox to import all homozygous reference positions, 0/0. Clear the checkbox to omit homozygous reference positions from the import.

Modify Table Views

To modify how data appear in the tables, click the column headings. Options include sorting in descending or ascending order, showing only selected data based on listed values, or adjusting column order.



NOTE

Modifying how data appear in the Variants table only affects how information is arranged in the table. Modifying views does not change the underlying data.

Sort Data in Ascending or Descending Order

To change the order in which data appear in the Variants table, click a column heading. Data are sorted in either descending or ascending order of values listed in that column. Click again to reverse the order.

- ▶ When the table is sorted in ascending order, an up arrow appears in the column heading.
- ▶ When the table is sorted in descending order, a down arrow appears in the column heading.

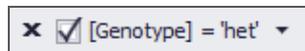
Show Only Selected Data

To show only selected data based on information in the Variants table, use the show/hide  icon in the column heading.

- 1 Click the show/hide  icon in the column heading. A drop-down list opens that contains all values present in that column.
- 2 Select a value from the drop-down list. The Variants table shows only data that contains your selection.

To restore the default view of the Variants table, use 1 of the following methods:

- ▶ Click the show/hide  icon in the column heading used to modify the table and select **All** from the drop-down list.
- ▶ Click the  icon at the bottom of the Variants table.



A history of previous selections appears at the top of the column heading drop-down list for quick access to frequently used selections. To remove a selection from history, click the delete  icon.

Show or Hide Selected Columns

Use the checkboxes below the table tabs to show or hide groups of columns in the Variants table. Select the checkbox to show data, and clear the checkbox to hide data.

Show/hide options include the following sections of the Variants table:

- ▶ **Show Population Frequencies**—Shows and hides Allele Freq, Allele Freq Global Minor, global Minor Allele, Allele Freq Amr, Allele Freq Eas, Allele Freq Sas, Allele Freq Asn, Allele Freq Af, Allele Freq Eur, and Allele Freq EvsEVS Freq All, ExAC Freq All, ExAC Freq Afr, ExAC Freq Amr, ExAC Freq Eas, ExAC Freq Sas, ExAC Freq Fin, ExAC Freq Nfe, ExAC Freq Other, ExAC Freq Amr.

- ▶ **Show Transcript Info**—Shows and hides Num Transcripts, Transcript, Consequence, cDNA Position, CDS Position, Amino Acids, Codons, Exon, Intron, Transcript HGNC, Canonical, Sift, PolyPhen, ENSP, HGVSc, and HGVSp.
- ▶ **Show Custom Annotations**—Shows and hides Custom Annotation, Custom Annotation 2, Custom Annotation 3, Custom Annotation 4, and Custom Gene Annotation.
- ▶ **Show ClinVar**—Shows and hides ClinVar RS, ClinVar Alleles, ClinVar Allele Type, ClinVar Significance, ClinVar Disease Name, ClinVar Accession, ClinVar MedGen, ClinVar OMIM, ClinVar Orphanet, ClinVar Gene Reviews, and ClinVar SnoMedCt ID.
- ▶ **Show COSMIC**—Shows and hides COSMIC ID, COSMIC Allele, COSMIC Gene, COSMIC Primary Site, and COSMIC Histology.

Set Table Options

The Table Options menu includes the following commands:

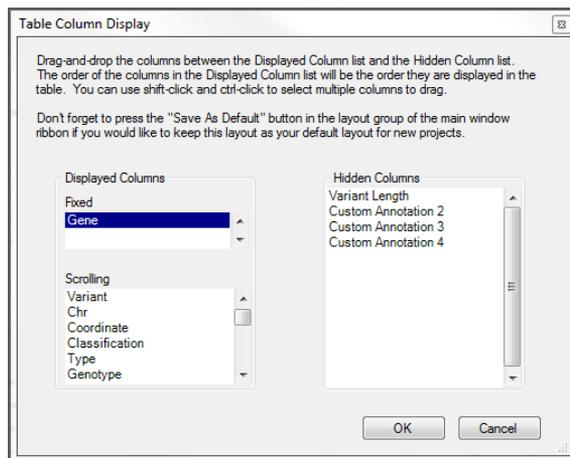
- ▶ **Select All**, which selects all rows in a table.
- ▶ **Copy**, which copies selected data to the clipboard.
- ▶ **Smaller** and **Larger**, which changes text size in a table.
- ▶ **Column Order**, which includes commands to change table layout.

From the Table Options menu, click **Column Order** to open the Table Column Display window. From this window, drag and drop column headings to specify table layout:

- 1 To show or hide columns, drag and drop column headings from the Displayed Columns list to the Hidden Columns list.
- 2 To prevent selected columns from scrolling horizontally, drag and drop column headings from the Scrolling list to the Fixed list.
- 3 Click **OK** when you are finished.
- 4 To save this layout for use in other VariantStudio projects, click **Save As Default** from the Layout menu.

In the following example, the Gene column is set to Fixed, and variant length and optional custom annotation columns are hidden.

Figure 6 Table Column Display Window



Applying Annotations and Classifications

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Annotate Variants

From the Annotations and Classification tab, use commands on the Annotate menu to annotate variants in the current sample with options to annotate all variants or only those variants specified. All coordinates used in VariantStudio are genomic coordinates on the positive strand.

Always annotate variants before applying filters.

Command	Description
Annotate	Annotates variants in the project using the following options: <ul style="list-style-type: none"> • Current Sample— Annotates all variants in the current sample. The current sample is listed in the Current Sample field of the Samples menu. • All Samples— Annotates all variants within each sample imported into the project. This process can take time to complete depending on the number of samples in the project.
Custom Annotation	Imports and applies custom annotations. For more information, see <i>Input File for Custom Variant Annotations</i> on page 28.
Annotation Options	Specifies annotation transcript options. For more information, see <i>Annotation Options</i> on page 26.
Custom Gene Annotation	Imports and applies custom annotations. For more information, see <i>Input File for Custom Gene Annotations</i> on page 28.
Set Default Transcripts	Specifies the default transcript for each gene. For more information, see <i>Set Default Transcripts</i> on page 27.



NOTE

Although mitochondrial variants can be imported, the annotation database does not provide annotations for these variants.

Annotation Options

From the Annotate menu, click **Annotation Options**. The Annotation Options form opens with options to annotate only certain variants.

Option	Description
Transcript Annotation	Provides an option to annotate only variants in the canonical transcript, which is the longest translated transcript in the gene.
Transcript Source Type	Annotates variants identified in a specific annotation source, which is RefSeq by default. The default can be changed to Ensembl by editing the mode entry in the VariantStudio configuration file (VariantStudio.exe.config), as follows: <pre><add key="Mode" value="Ensembl"/></pre> Close and reopen VariantStudio to enable the change.

Set Default Transcripts

- 1 Click **Set Default Transcripts**. A window opens that lists the default transcript for each gene. By default, VariantStudio lists the canonical transcript, which is the longest translated transcript in the gene.
- 2 For genes with multiple transcripts, use the drop-down list to set the default to another transcript.

Alternatively, click **Browse** to navigate to a tab-delimited text file containing your preferred default transcripts and click **Load**.

Input File for Default Transcripts

The input file for default transcripts requires 2 columns: Gene_Name and Transcript_Name, as shown in the following example.

Gene_Name	Transcript_Name
ACTN3	NM_003793.3
ADH1B	NM_000668.4
AKAP10	NM_007202.3

Annotation Sources

Annotation sources are static in the VariantStudio software. Any changes to source databases, such as ClinVar, do not automatically update the annotation sources used by VariantStudio, which are stored locally on your computer.

The VariantStudio software uses the BaseSpace Annotation Engine (version 1.4.2.60) to obtain the annotations that are displayed in the variant grid. It includes annotations from the following annotation data sources:

- ▶ Variant Effect Predictor (VEP)
- ▶ 1000 Genomes Project
- ▶ Catalogue of Somatic Mutations in Cancer (COSMIC)
- ▶ ClinVar
- ▶ National Center for Biotechnology (NCBI)
- ▶ National Heart, Lung, and Blood Institute (NHLBI) Exome Variant Server
- ▶ UCSC

Create Custom Annotations

Applying custom annotations requires a tab-delimited input file with a *.txt extension. Variant-level annotations and gene-level annotations require different input file configurations.



NOTE

VariantStudio assumes that all annotations are expressed in the genomic coordinates on the positive strand, including any custom annotations that are imported into VariantStudio.

Input File for Custom Variant Annotations

The input file for custom variant annotations requires 5 columns: Chr, Position, Ref, Variant, and Annotation, as shown in the following example.

Chr	Position	Ref	Variant	Annotation
1	11046855	G	T	Good
1	11046909	A	T	Bad
1	14096821	T	C	Confirmed

- ▶ **Chr**—The chromosome for the variant (1–22, X, Y, or M).
- ▶ **Position**—The genomic coordinate of the variant on the chromosome (1-based).
- ▶ **Ref**—The reference base, or bases for an insert or deletion, at the specified position.
- ▶ **Variant**—The base, or bases for an insert or deletion, at the specified position.
- ▶ **Annotation**—The value assigned to a variant with matching values for chr, position, ref, and variant.
- ▶ [Optional] 3 additional annotation columns are recognized input for custom variant annotations. Use the headings **Annotation2**, **Annotation3**, and **Annotation4**.



NOTE

The Ref field and Variant field must be expressed in VCF format, where indels contain the preceding base in common between the reference and variant allele. For more information, see www.internationalgenome.org/wiki/Analysis/Variant_Call_Format/vcf-variant-call-format-version-41.

Input File for Custom Gene Annotations

The input file for gene annotations requires 2 columns: Gene and Annotation, as shown in the following example.

Gene	Annotation
AGRN	Myasthenia, limb-girdle, familial
CCDC39	Ciliary dyskinesia, primary, 14
DHTKD1	2-aminoadipic 2-oxoadipic aciduria

- ▶ **Gene**—The gene symbol.
- ▶ **Annotation**—The value assigned to the specified gene.

Apply Custom Annotations

- 1 Create a custom annotations file using a text editor, such as Notepad, and save it with a *.txt file extension.
- 2 From the Annotate menu, click **Custom Annotations** to apply annotations to variants or **Custom Gene Annotations** to apply annotations to genes.

- 3 Browse to the custom annotations file and click **OK**. This step links the custom annotations file to the project.
- 4 Use the custom filters in the Filters pane to filter data based on custom annotations. For more information, see *Custom Filters* on page 40.

Apply Variant Classifications

Introduced in VariantStudio v2.1, you can apply classifications to variants according to their biological impact. Classifications are stored in the classification database.

Command	Description
Apply Classifications from Database	Use this command to apply classifications to any variants in the current sample that are listed in the classification database. For more information, see <i>Apply Classifications from Database</i> on page 30.
View Classification Database	Use this command to open the classification database, edit entries in the database, or import classifications from an external file. For more information, see <i>View Classifications Database</i> on page 33.
Classification Settings	VariantStudio provides 5 classifications: Benign, Presumed Benign, Presumed Pathogenic, Pathogenic, and Unknown Significance. Use this command to add or remove classification categories. For more information, see <i>Add or Remove Classification Categories</i> on page 33.

Variant classifications can be changed at any time in the classification database or changed locally in the current project without changing database entries. For more information, see *Edit Variant Classifications* on page 32.

By default, the classification database is saved locally for use with any VariantStudio project that is opened locally. For more information, see *Classification Database Location* on page 33.

A backup of the classification database is created with the first change of each day. For more information, see *Classification Database Backup* on page 33.

For each classified variant, the following text fields are available for recording comment.

- ▶ **Notes**—Information in Notes field is stored in the classification database only.
- ▶ **Report Fragment**—Information in the Report Fragment field is stored in the classification database and exported as a column in the sample report. For more information, see *Sample Report Overview* on page 53.

There are 3 ways to apply classifications to variants in a project:

- ▶ From the menu, apply classifications to variants in the current sample that are listed in the classification database.
- ▶ From the Variants table, apply a classification to a selected variant in the Variants table and save the classification to the database.
- ▶ From the Variants table, apply a classification to multiple selected variants in the Variants table and save the classification to the database. The same classification must apply to all selected variants.

Apply Classifications from Database

- 1 Click **Apply Classifications from Database**.
Any variants in the current project that have matching criteria in the classification database are annotated with the classification as specified in the database.

Apply Classifications in the Variants Table

- 1 Click the  icon in the Classification column for the variant you want to classify. The Classification for Variant in Database window opens, which shows information for the variant and provides a drop-down list of available classification categories.
- 2 Select a classification category from the Classification drop-down list, and enter any applicable comments in the Notes field and Report Fragment field.
- 3 Click **Save Changes to Database**. The classification can later be removed from the classification database.

Apply Classifications to Multiple Variants

- 1 Use shift-click or ctrl-click to select more than one row in the Variants table.
- 2 Right-click in the Classifications column over a selected row, and then select **Classify Selected Variants**.
- 3 From the Classify Selected Variants window, use the drop-down list to assign a classification. Enter any applicable comments in the Notes field and Report Fragment field.
- 4 Click **OK**. The classification assignments are saved to the database automatically.

Edit Variant Classifications

There are 2 ways to edit classifications for variants with assigned classifications:

- ▶ Edit variant classifications in the database.
- ▶ Edit classifications locally in the current project without changing database entries.

Edit Classifications in the Database

- 1 Click the  icon in the Classification column for the variant you want to change. A window opens that shows information about the variant, the current classification, and any comments in the Report Fragment field.
- 2 Click **Edit Classification in Classification Database**. The Classification for Variant in Database window opens.
- 3 Do 1 of the following:
 - ▶ To change the classification in the database, select a new classification from the Classification drop-down list and click **Save Changes in Database**.
 - ▶ To remove the classification from the database, click **Remove Classification from Database**.

Edit Variant Classifications Locally

To change variant classifications locally, the variant must already have a classification assigned in the classification database.

- 1 Click the  icon in the Classification column for the variant that you want to edit locally. A window opens that shows information about the variant, the current classification, and any comments in the Report Fragment field.
- 2 Select a different classification category from the Classification drop-down list.
- 3 Click **OK**. The classification is applied to the variant in the current project only. The variant classification recorded in the database can be reapplied to the variant later.
- 4 To revert the classification to what is assigned in the database, click the  icon in the Classification column.
- 5 Click **Apply Classification from Classification Database**. The classification recorded in the database appears in the Classification field. Click **OK**.

Manage Classifications

From the Annotations and Classification tab, use commands on the Classifications menu to view the classification database and manage classification settings.

View Classifications Database

- 1 Click **View Classification Database** to view the entries in the classification database. From this window, you can edit an entry, delete an entry, or import classifications from an internal file.
 - ▶ **Edit an entry**—Select a row, or use shift-click or ctrl-click to select multiple rows. Click **Edit Selected**. Reassign a classification or add comments.
 - ▶ **Delete an entry**—Select a row, or use shift-click or ctrl-click to select multiple rows. Click **Delete Selected**. The entry is permanently deleted from the database.
 - ▶ **Import classifications**—Click **Import Classifications** and browse to the location of your external classifications file. For more information, see *Import Classifications* on page 34.

Add or Remove Classification Categories

- 1 Click **Classification Settings** to add, remove, or rename classification categories.
 - ▶ **Add**—In the Add Category field, enter a new category name. Click **Add**.
 - ▶ **Remove**—Select a category from the list. Click **Remove**.
 - ▶ **Rename**—Select a category from the list. Click **Rename** and enter a new name.

Classification Database Location

By default, the classification database is saved locally in
C:\ProgramData\Illumina\Illumina VariantStudio\ClassificationDb.bin.

When the database is stored locally, classifications are available for the current project and any future projects opened on that computer.

If the classification database is stored on a network location, classifications are available to projects opened in any installation of VariantStudio with access to that network location.

- 1 To change the default setting, open the VariantStudio configuration file in C:\Program Files\Illumina\Illumina VariantStudio\VariantStudio.exe.config.
- 2 In the value field of the **ClassificationDatabaseFilePath** key, enter the preferred network path.
- 3 Save and close the configuration file.
- 4 Close and reopen VariantStudio to enable the change.

Classification Database Backup

A backup of the classification database is created the first time the database is changed on any given day.

The backup is named DDMMYYYY.bin and is stored in the folder DatabaseBackups, which is located in the same folder as the classification database, C:\ProgramData\Illumina\Illumina VariantStudio\DatabaseBackups.

Import Classifications

To import classifications to the classification database from an external file, create an input file in a tab-delimited text format (TSV) using a *.tsv file extension. From the Classification menu in the Annotations and Classification tab, select **View Classification Database**, then select **Import Classifications**.

The input file requires 5 columns: Chr, Position, Ref, Variant, and Classification, as shown in the following example. Optionally, include a Notes column and a Fragment column.

Chr	Position	Ref	Variant	Classification	Notes
1	11046855	G	T	Classification 1	Note 1
1	11046868	C	G	Classification 2	Note 2
1	11046909	A	T	Classification 3	Note 3

- ▶ **Chr**—The chromosome for the variant (1–22, X, Y, or M).
- ▶ **Position**—The genomic coordinate of the variant on the chromosome.
- ▶ **Ref**—The reference base, or bases, for an insert or deletion at the specified position.
- ▶ **Variant**—The base, or bases, for an insert or deletion at the specified position.
- ▶ **Classification**—The value assigned to a variant with matching values for chr, position, ref, and variant. The classification name must match 1 of the classifications listed in your database.
- ▶ **Notes**—Note about the entry. Information in this field is not included in the sample report.
- ▶ **Fragment**—Notes about the entry that are intended for the sample report.



NOTE

Make sure that you add any new classification names to the database using the Classifications Settings command.

Best Practices for Importing Classifications



NOTE

All coordinates used in VariantStudio are genomic coordinates on the positive strand.

Before importing previously classified variants into the VariantStudio classification database, convert classifications to genomic coordinates. This step is especially important for variants that were classified based on HGVS notations and transcripts.

Importing classifications before converting to genomic coordinates can result in some variants not being annotated with imported classifications when you use the command Apply Classifications from Database. Because VariantStudio assumes that all annotations are expressed in genomic coordinates on the positive strand, the classification database requires an exact match for variants to be annotated with stored classifications.

Applying Filters

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Apply Filters

The Filters pane provides options for applying any combination of filters to the data in your project. Filters are grouped in 9 expandable sections: General, Variant, Gene, Consequence, Population Frequency, Cross Sample Subtraction, Family Based, Custom, and Classification.

- 1 Click the down arrow  icon to expand a filter section.
- 2 From the available options, select filter settings. Use any combination of settings from any number of filters.
- 3 Click **Apply Filters**. Filters are applied to the current sample only, not to all samples that are imported into the project.
- 4 Click **Clear Filters** to remove applied filters.



NOTE

You can create a filter using any combination of the filter options in the Filters pane, and then save the combination as a single filter. Saved filters can later be applied to other samples. For more information, see *Create Favorite Filters* on page 48.

General Filters

Use the General filters to filter data by genotype, variant type, and chromosome.

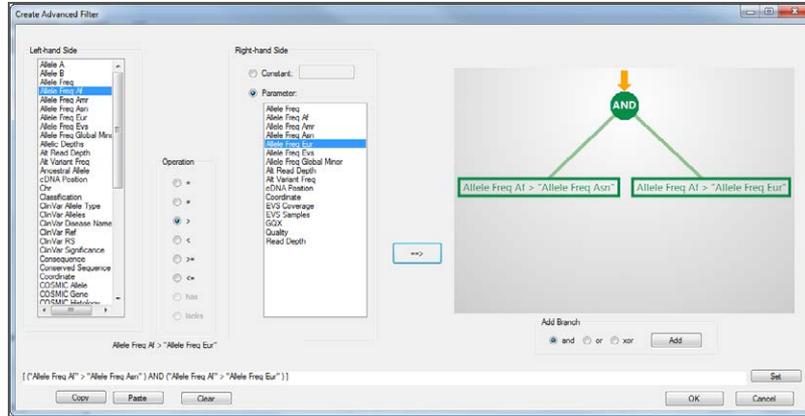
Filter Name	Setting Description
Genotype	Filters data to show any combination of heterozygote, homozygote, or hemizygote. All options are selected by default.
Variant Type	Filters data to show any combination of SNVs, Insertions, Deletions, or Reference calls.
Chromosome	Filters data to show all chromosomes (default), autosomal chromosomes, or a specific chromosome number.
Advanced	Filters data based on selections that you make in the Advanced Filter window.

Advanced Filter Options

Use the Advanced filter options to create a multibranch Boolean expression for filtering data in the Variants table. As you build the advanced filter, a diagram appears to illustrate the filter and branches in the expression.

- 1 Select the checkbox labeled **Use Advanced Filter** and then click **Edit Filter**. The Create Advanced Filter window opens.

Figure 7 Create Advanced Filter Window



- 2 Select a column heading from the list on the left-hand side.
- 3 Select an operation from the Operation list.
- 4 Select either **Constant** or **Parameter**.
 - ▶ To filter on a constant, enter a constant associated with the selection from the left-hand column.
 - ▶ To filter on a parameter, select a column heading from the list on the right-hand side.
- 5 Click the generate filter  button. A diagram of the filters appears.
- 6 To add another branch to the advanced filter, select the radio button for either **and**, **or**, or **xor** (exclusive). Then, click **Add**. A new branch is added to the diagram.
- 7 Continue selecting options and operators until you have completed the filter.
- 8 [Optional] To delete a branch, right-click the branch, then select **Delete**.
- 9 When the advanced filter is complete, click **OK**.
- 10 From the Filters pane, click **Apply Filters**.

Variant Filters

Use the Variant filters to filter by variant call attributes, variant positions, and variants with specific annotation.

Filter Name	Setting Description
Variant Call	Filters data based on a specified value for variant call quality: pass filter, quality score, read depth, or percentage of variant frequency for the minor allele. Select the checkbox, and then use the up/down arrows to specify a minimum threshold.
Show only variants	Filters data based on variant position: <ul style="list-style-type: none"> • Inside genes • In conserved regions

Filter Name	Setting Description
Only variants with	Filters data based on the source of annotation. Options include variants without dbSNP ID, with COSMIC annotation, and with ClinVar annotation. COSMIC and ClinVar annotation enable 2 more choices: <ul style="list-style-type: none"> • If <i>where matches mutant allele</i> is selected and variant has multiple records, only pass variant if at least one matches. • If <i>where not matches mutant allele</i> is selected and variant has multiple records, only pass variant if none matches.

Gene Filters

Use the Gene filters to filter data by disease, or include or exclude specific genes.

Filter Name	Setting Description
Disease	Filters data to show genes associated with the specified disease. Enter the disease name. This field is not case-sensitive.
Include List	Filters data to include specified genes. To include genes, click the  button to open the Edit Included Gene List field, and enter the gene name. This field is not case-sensitive.
Exclude List	Filters data to exclude specified genes. To exclude genes, click the  button to open the Edit Excluded Gene List field, and enter the gene name. This field is not case-sensitive.
Min Variant Alleles	Filters data to show only variants that overlap genes with the specified number of variant alleles. A homozygous variant counts as 2 variant alleles, while a heterozygous variant counts as 1 variant allele.
Custom and Optional Gene Annotation	Filters data to show only genes with a specified custom annotation. You can use Boolean logic (AND and OR) to between the Custom and Optional Gene Annotation filters. The Optional Gene Annotation filter is based on the optional second annotation column that you can import.



NOTE

If you click **Clear Filters**, the gene list is also cleared. To save a gene list, create a favorite filter. For more information, see *Create Favorite Filters* on page 48.

Consequence Filters

Use the Consequence filters to filter data by variants that alter the coding potential of the transcript.

- 1 Select the checkbox **Show only variants that are**.
- 2 Select the checkbox for each individual consequence setting or click **Select All**.

Filter Name	Setting Definition
Missense	A single base pair substitution that results in the translation of a different amino acid at that position. Note: PolyPhen and SIFT report only SNVs.
PolyPhen, damaging	A prediction of a damaging effect of an amino acid substitution on the function of a human protein based on PolyPhen.
SIFT, deleterious	A prediction of a deleterious effect of an amino acid substitution on the function of a human protein based on SIFT.
Frameshift	An insertion or deletion involving a number of base pairs that is not a multiple of 3, which disrupts the triple reading frame.
Stop gained	The gain of a stop codon in the coding sequence.
Stop loss	The loss of a stop codon in the coding sequence.
Initiator codon	A codon that acts as a start signal for the synthesis of a protein.
Inframe insertion	An insertion that does not alter the reading frame as a result of the insertion.
Inframe deletion	A deletion that does not alter the reading frame as a result of the deletion.
Splice	An insertion, deletion, or substitution that occurs in a splice region of the gene. A splice is not in a coding region.

Population Frequency Filters

Use the Population Frequency filters to filter data based on the allele frequency in population studies.

Options include global frequency, American, East Asian, South Asian, African, European, European (Finnish), European (non-Finnish), Other, and EVS.

- ▶ American, East Asian, South Asian, and African are allele frequency from 1000 Genomes or ExAC.
 - ▶ European is allele frequency from 1000 Genomes.
 - ▶ European (Finnish), European (non-Finnish), and Other are allele frequency from ExAC.
 - ▶ EVS is allele frequency from the NHLBI exome sequencing project.
- 1 Select the checkbox and then use the up/down arrows to specify a value expressed as percentage.
 - 2 To set the same value to all populations, use the up/down arrows in the **Set all to** field. Click **Set All**.

Cross Sample Subtraction Filter

If multiple samples are present in the project, use the cross sample filter to exclude variants that are also present in another sample.

- 1 Select the checkbox **Use Cross Sample Subtraction**.

- From the drop-down list, select a sample in the project. Only one sample can be selected as the cross sample filter.

This filter is helpful when filtering variants present in tumor-normal samples.

Family Based Filter

Use the Family Based filter to filter for variants that are consistent with user-specified inheritance mode and provided variant data for available family members. The Family Based Filter requires input of at least one parent or sibling. This filter is useful in identifying candidate disease causing variants.



NOTE

Family Based filters are not available when View All Samples is selected.

- Select the checkbox **Use Family Based Filtering**.
- Using the Type drop-down list, select a type from the following choices:
 - ▶ **X-linked Recessive**—Variant-level filtering of heterozygous variants in affected females that are not present in the father and hemizygous in affected males.
 - ▶ **Autosomal Recessive transmission**—Gene-level filtering of different heterozygous variants in the same gene in relatives, or variant-level filtering of the same heterozygous variants in both parents.
 - ▶ **De novo mutation**—Filters variants not present in the relatives. This filter can also be applied using the cross-sample subtraction filter.
 - ▶ **Autosomal Dominant transmission**—Variant-level filtering of heterozygous variants that are present in the affected relatives, and not present in the unaffected relatives. This filter requires that you indicate the affected relatives.
- Use the drop-down lists to select at least one parent or sibling. The child sample is set to the current sample by default, and all samples to be used in the family-based filtering must be present in the current project.

Best Practices When Using the Family Based Filter

When using gVCF files for family-based filtering, variants that were not called in the parents are included if the variants are otherwise consistent with the selected inheritance mode.

If you have a gVCF file, use a gVCF viewer such as the Integrative Genomics Browser (IGV) to examine the no coverage regions. Check for the presence of a disease gene of interest in samples from the child and other family members. For more information, see www.broadinstitute.org/igv/.

Custom Filters

Custom filters enable filtering based on input provided in the custom annotations input file. For more information, see *Create Custom Annotations* on page 28.

Filter Name	Setting Description
Do not filter on custom annotation	Turns off custom annotations. This setting is on by default.

Filter Name	Setting Description
Show variants with annotation	Filters data to show variants that match criteria provided in the custom annotations input file with an assigned annotation value in the annotations column.
Show variants without annotation	Filters data to show variants that match criteria provided in the custom annotations input file without an assigned annotation value in the annotations column.
Show variants that contain	Filters data to show variants that match criteria provided in the custom annotation input file. Options include annotations from any of the 4 possible annotation columns.

Classification Filter

Use the classification filter to filter by classifications assigned in the classification database. Any customized classifications appear in the classification filters list.

- 1 Select the **Filter by classification** checkbox.
- 2 Select the checkbox next to any number of available classifications.

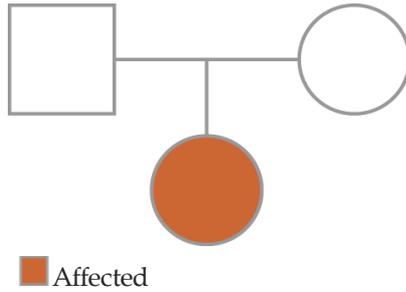
Family-Based Filtering Workflows

The mode of inheritance, which is the inheritance pattern of a genetic trait or disorder as passed down through generations, is typically 1 of the following:

- ▶ Autosomal recessive
- ▶ Autosomal dominant
- ▶ X-linked recessive
- ▶ *De novo* mutation

Disease-causing variants coexist with the disorder according to the mode of inheritance. Family-based filtering requires at least 2 samples, the affected person (proband), and at least 1 parent or sibling.

Figure 8 Example: Father, Mother, and Proband

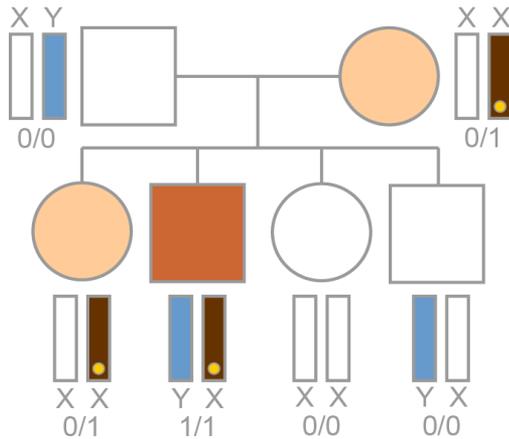


Because the proband contains thousands of variants that appear deleterious, filtering is necessary to remove variants unlikely to cause disease and identify possible disease-causing variants.

X-Linked Recessive Transmission Workflow

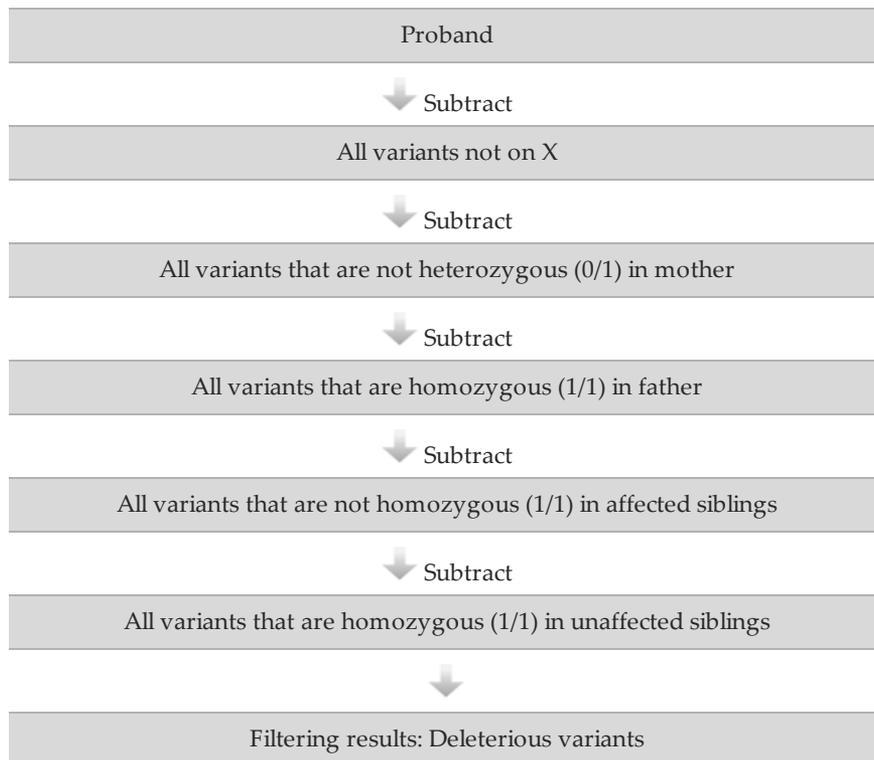
- ▶ A variant is on the X chromosome
- ▶ The variant is heterozygous (0/1) in the mother
- ▶ The variant is not present in the father
- ▶ The variant is homozygous (1/1) in the affected child

Figure 9 X-Linked Recessive Transmission Logic



- Unaffected reference
- Unaffected carrier
- Affected
- Mutation

X-Linked Recessive Transmission Workflow

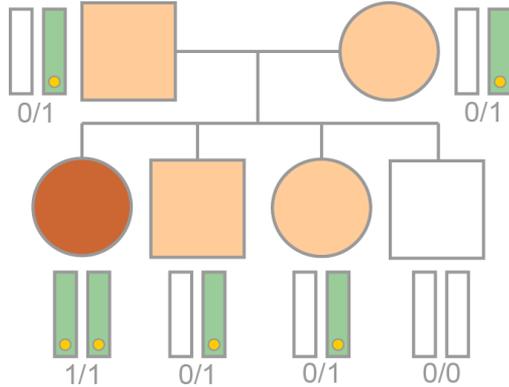


Autosomal Recessive Transmission Workflow

Recessive transmission has 2 possibilities.

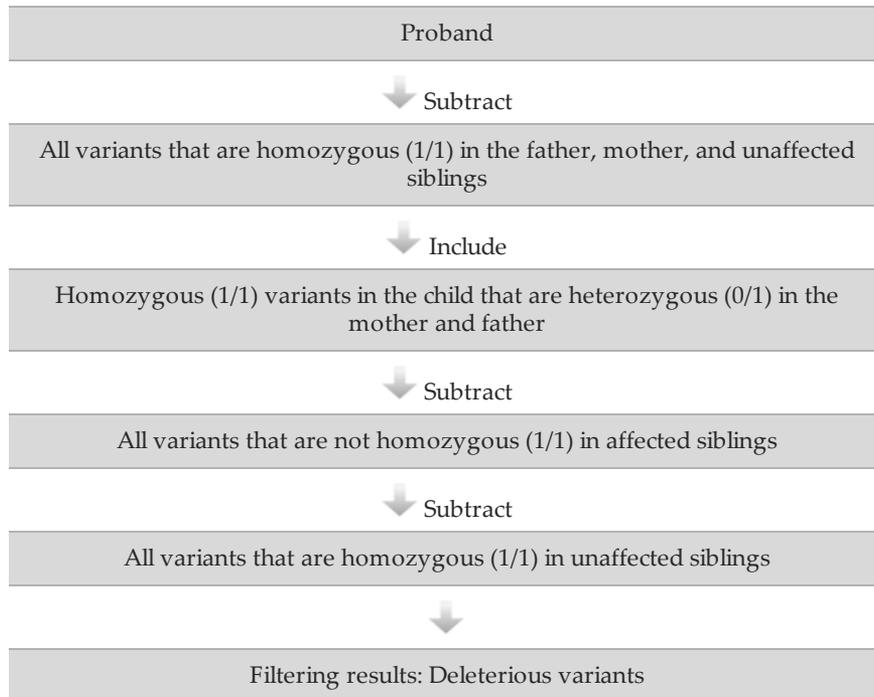
- ▶ A single gene contains a variant that is:
 - ▶ Heterozygous (0/1) in the mother
 - ▶ Heterozygous (0/1) in the father
 - ▶ Homozygous (1/1) in the affected child

Figure 10 Autosomal Recessive Transmission Logic #1



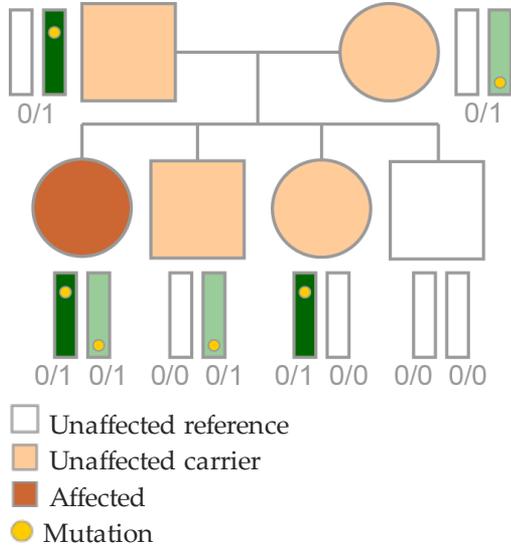
- Unaffected reference
- Unaffected carrier
- Affected
- Mutation

Autosomal Recessive Transmission Workflow #1

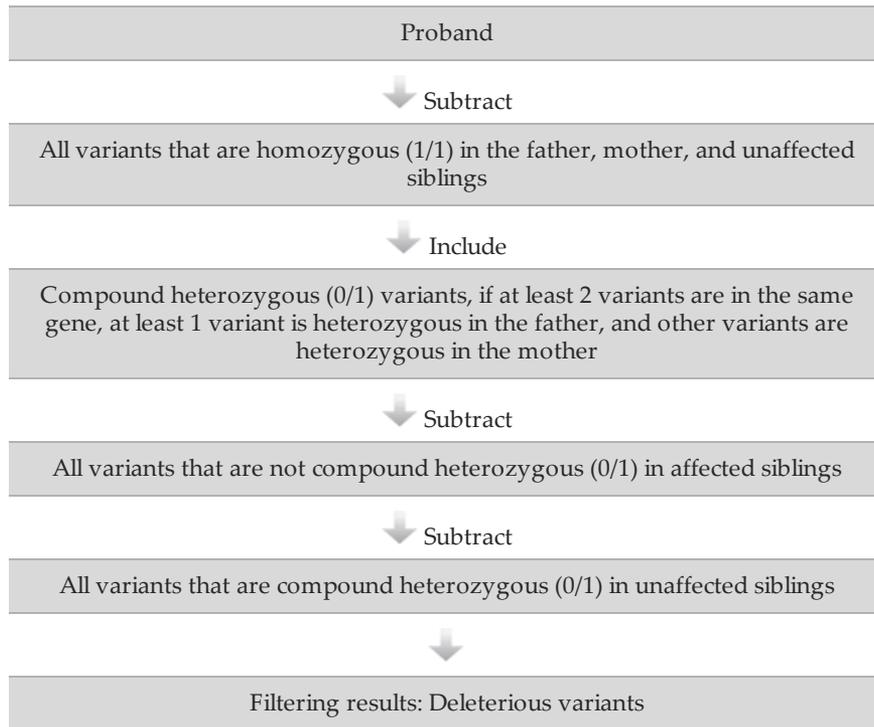


- ▶ A single gene contains:
 - ▶ One variant that is heterozygous (0/1) in the mother
 - ▶ The same gene contains a different variant that is heterozygous (0/1) in the father
 - ▶ Both variants are present (0/1 and 0/1) in the affected child

Figure 11 Autosomal Recessive Transmission Logic #2



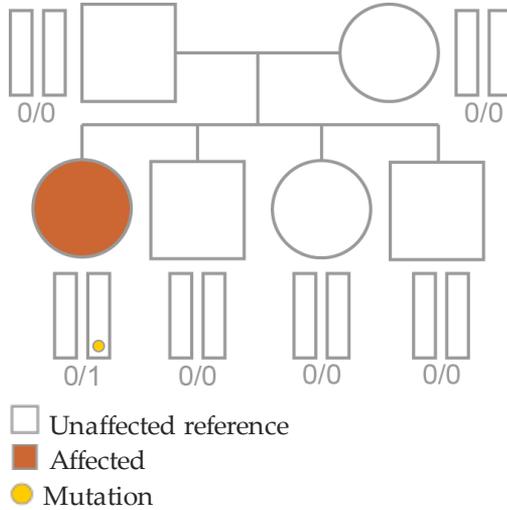
Autosomal Recessive Transmission Workflow #2



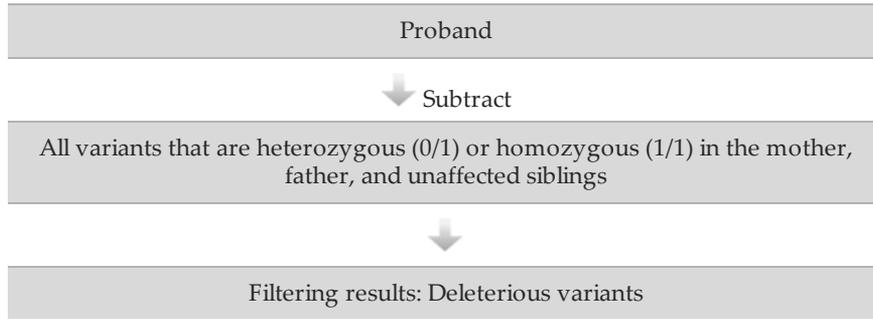
De Novo Mutation Workflow

- ▶ A variant is present (0/1 or 1/1) in the proband
- ▶ The variant is not present (0/0) in family members
- ▶ Only 1 child in the family is affected

Figure 12 De Novo Mutation Logic



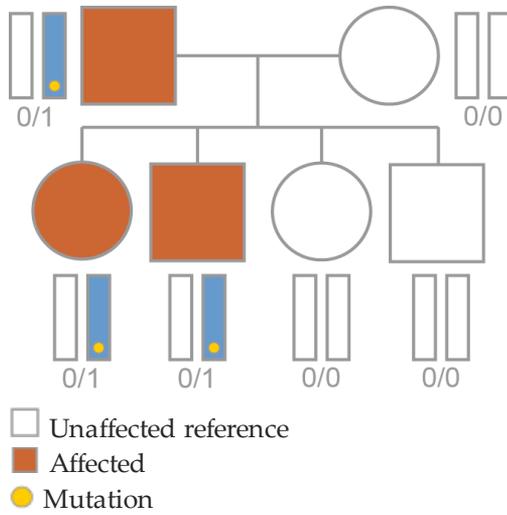
De Novo Mutation Workflow



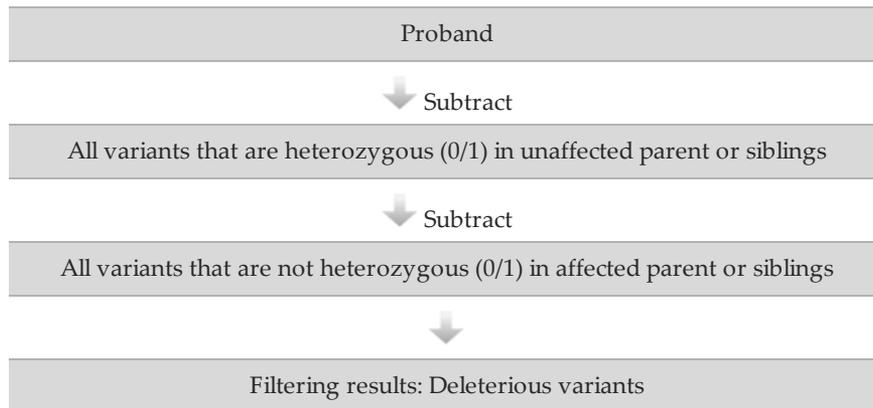
Autosomal Dominant Transmission Workflow

- ▶ A variant is heterozygous (0/1) in the affected parent
- ▶ The variant is not present (0/0) in the unaffected parent
- ▶ The variant is heterozygous (0/1) in the affected children

Figure 13 Autosomal Dominant Transmission Logic



Autosomal Dominant Transmission Workflow



Create Favorite Filters

To save any combination of filtering options for use with a different sample or for later use in another project, save the filtering options as a favorite filter.

The Filter Favorites menu includes commands to save, apply, modify, and manage saved filters.

Command	Description
Current	Shows the current filter that is applied and a list of available saved filters. Select a saved favorite filter from the drop-down list to apply it to the current sample. If you change to another sample, a favorite filter applied to the previous sample is not applied automatically to the next sample.
Manage Filters	Opens tools for renaming, duplicating, or deleting saved filters.
Save	Saves changes to the currently applied filter.
Save As	Opens a dialog box for naming a favorite filter.

Save a Favorite Filter

- 1 With any combination of filters specified in the Filters pane, select **Apply Filter**.
- 2 Click **Save**  in the Filter Favorites menu.
- 3 Enter a name for the new filter, and then click **OK**. When a saved filter is applied, the saved filter name appears in the Current field.

Apply a Favorite Filter

- 1 To apply a saved filter, expand the Current field drop-down list.
- 2 Select a filter name from the list. The filter is applied automatically.
- 3 To change to another saved filter, expand the drop-down list in the Current field, and select a different filter name.
- 4 Alternatively, click the blank entry at the top of the saved filters list to remove the currently applied filter. The variants table is restored to an unfiltered view.



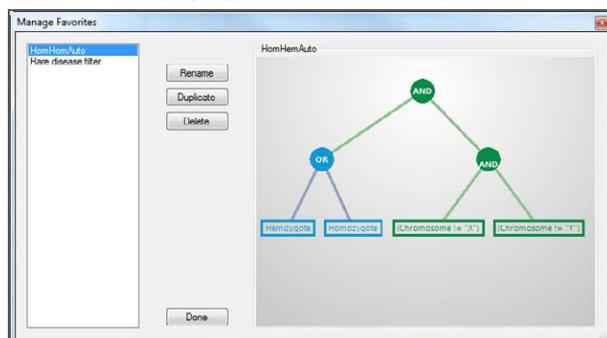
NOTE

The favorite filter is not automatically applied when you move to another sample in your project. To apply a favorite filter, reselect the favorite filter name from the Current drop-down list.

Manage Favorite Filters

To rename, duplicate, or delete favorite filters, use the Manage Favorites feature.

Figure 14 Managing Favorite Filters



- 1 Click **Manage Favorites**. Names of saved filters appear on the left panel and a block diagram of the selected filter appears on the right panel. To adjust the view of the block diagram, click anywhere on the right panel and use the scrolling feature on your mouse to zoom in or zoom out.
- 2 Click **Done** to apply changes.

Generating Reports

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Generate Reports

VariantStudio provides tools to export results from a project to an external report. Use the commands on the Reports tab to create a sample report, and to export data to text files and graphical representations.

Sample Report Overview

The sample report consists of 5 sections plus a footer and is generated as a PDF file or RTF file, depending on your preference. A sample report includes the following sections:

- ▶ **Lab information**—Typically, this section is defined in the template and appears as a header in the sample report.
- ▶ **Sample information**—This section contains details about the sample and appears as a two-column table in the sample report. The first column contains the field name and the second column contains the value. There are 2 ways to populate this section of the report:
 - ▶ Specify field names using the Manage Templates feature and then manually enter the value in the text fields.
 - ▶ Import sample information from an external text file. The text file must have 2 tab-delimited or comma-separated columns, 1 for the field name and 1 for the field value, and use a *.txt, *.csv, or *.tsv extension.
- ▶ **Test summary**—This section is reserved for a description of the test performed. Set up preferred content and formatting in the template. Add information specific to the report when you create the report.
- ▶ **Results**—This section lists variants in the open project that have a classification assigned. This section is blank if the project does not contain assigned classifications.
- ▶ Results are formatted in a four-column table with headings of Gene, Variant, Classification, and Details. Information in the Details column comes from the Report Fragment field in the classification database.

Figure 15 Example of a Results Section

Gene	Variant	Classification	Details
C3	NM_000064.2:c.941C>T	Benign	
GHRHR	NM_000823.3:c.169G>A	Benign	
GHRHR	NM_000823.3:c.363G>T	Benign	
ABCC9	NM_020297.2:c.1165-6delT	Presumed Benign	
C3	NM_000064.2:c.304C>G	Presumed Benign	
APOE	NM_000041.2:c.388T>C	Pathogenic	
CASP10	NM_032977.3:c.1228G>A	Pathogenic	
CLCN1	NM_000083.2:c.2680C>T	Pathogenic	
NCF1	NM_000265.4:c.73_74delGT	Pathogenic	
POLG	NM_002693.2:c.1399G>A	Pathogenic	
SLC4A1	NM_000342.3:c.166A>G	Pathogenic	
BCHE	NM_000055.2:c.1699G>A	Unknown Significance	
MEFV	NM_000243.2:c.1772T>C	Unknown Significance	

- ▶ **Methodology**—This section is reserved for a description of the methodology specific to the report. Set up preferred content and formatting in the template. Add information specific to the report when you create the report.
- ▶ **References**—This section is reserved for references applicable to the contents of the report.
- ▶ **Page footer**—Typically, this section is defined in the template. For example, the footer can contain the facility address and contact information, or it can be blank.

Create a Sample Report Template

Use the Manage Templates feature to create a customized template for sample reports. After templates are created, use Manage Templates to duplicate, edit, rename, or delete templates in the template library.

VariantStudio includes an example report template to help in creating a template. The example report template cannot be edited. Instead, create a copy of the example report template. From this copy, rename the template and customize each section of the template using the template tabs.

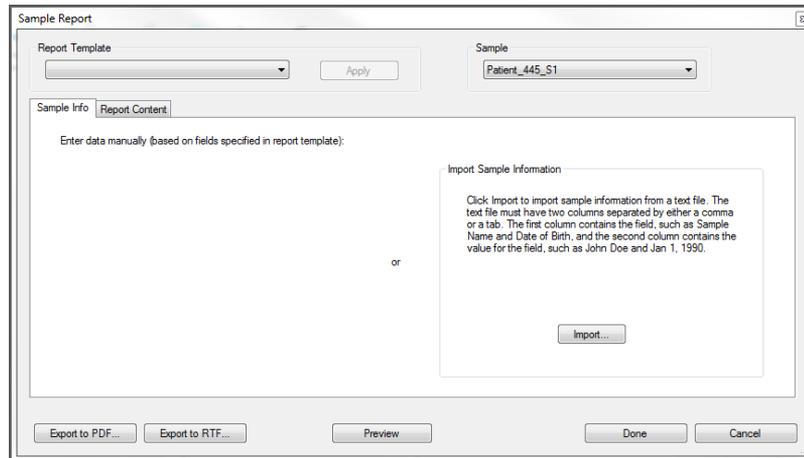
- 1 Click **Manage Templates**. The Manage Report Templates window opens.
- 2 Do 1 of the following:
 - ▶ Highlight Example Template in the Report Template field, then click **Duplicate** and enter a template name. Click **OK**.
 - ▶ To create a template without using the example template, click **New** and enter a template name. Click **OK**.
 - ▶ To edit an existing template, click to highlight the template name in the Report Template list. This template is now the active template and ready for editing.
- 3 For each of the following tabs, enter the information to be included in reports using this template. Use the formatting tools to customize the layout. Information included in the following sections of the template are editable when creating the sample report.
 - ▶ **Sample Info tab**—Specify the sample fields to include in the report. Each field name generates a row in the sample information table.
 - ▶ **Lab Information tab**—Enter the lab name and location, or other preferred information for the report header.
 - ▶ **Test Summary tab**—Enter preferred introductory content to begin this section. Otherwise, leave this section blank in the template.
 - ▶ **Methodology tab**—Similar to Test Summary, enter preferred introductory content to begin this section. Otherwise, leave this section blank in the template.
 - ▶ **References tab**—Similar to Test Summary, enter preferred introductory content to begin this section. Otherwise, leave this section blank in the template.
 - ▶ **Page Footer tab**—Enter preferred content for the template footer, such as contact information. This information appears at the bottom of each page in the report.
- 4 Click the **Classifications** tab. Drag and drop classification names from the Available Classification list to the Displayed Classification list. The selected classifications are included in any reports using this template and they appear in the order listed.
- 5 Click **Save Changes**.
- 6 [Optional] With the template name highlighted in the Report Template list, click **Make Default**. The current default is listed in the lower-left corner of the Manage Report Templates window.
- 7 Click **Done**. The Manage Report Templates window closes.

Create a Sample Report

Before proceeding, consider creating a template using the Manage Templates feature. For more information, see *Create a Sample Report Template* on page 54.

- 1 From the Reports menu, click **Sample Report**. The Sample Report window opens.

Figure 16 Sample Report, Sample Info Tab



- 2 From the Report Template drop-down list, select an appropriate template for the report, and then click **Apply**.
- 3 From the Sample drop-down list, select a sample.
- 4 On the Sample Info tab, enter information in the fields provided or click **Import** to browse to the location of the text file containing the information.
- 5 Enter information for the remaining tabs that are not already populated in the selected template.
- 6 Click **Preview** to preview the report before generating it.
- 7 [Optional] Click **Done** to save the report contents without generating the report.
- 8 Click **Export to PDF** or **Export to RTF** to generate the report.

Export Text Files and Charts

In addition to sample reports, VariantStudio provides tools for exporting to text files and graphical representations of data.

Export Data Files

From the Export menu in the Reports tab, select an option to export transcript or variant data to a tab-separated values file. These text file formats are not application-specific and can be opened in any text editor.

Command	Description
Filtered Variants (TSV)	Exports filtered variants from the current sample. For variants that overlap multiple genes, only the transcripts that appear on the interface are exported.
All Transcripts for Variants (TSV)	Exports all transcripts for filtered variants in the sample.
Filter History (CSV)	Exports a report of all filters applied to the project.

Export Charts

- From the Charts menu, select a preferred format to export results in a histogram or a pie chart.
 - ▶ **Histogram**—Generates a histogram of filtered results from the Variants table.
 - ▶ Use the Plot drop-down list on the generated histogram (Figure 17) to represent variant quality values or indel variant length.
 - ▶ Use Data Source options to show all variants or only filtered variants.
 - ▶ **Pie Chart**—Generates a pie chart of filtered results from the Variants table.
 - ▶ Use the Plot drop-down list on the generated pie chart (Figure 18) to represent percentages of variant call filters, variant type, genotype, coding regions, or filtered variants.
 - ▶ Use Data Source options to show all variants or only filtered variants.
- From the generated chart, click **Copy to Clipboard** to transfer the image from the VariantStudio software to an application that supports images.

Figure 17 Histogram

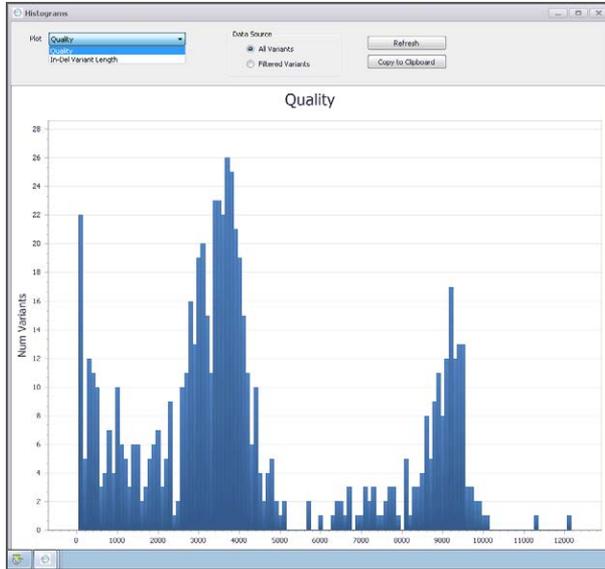
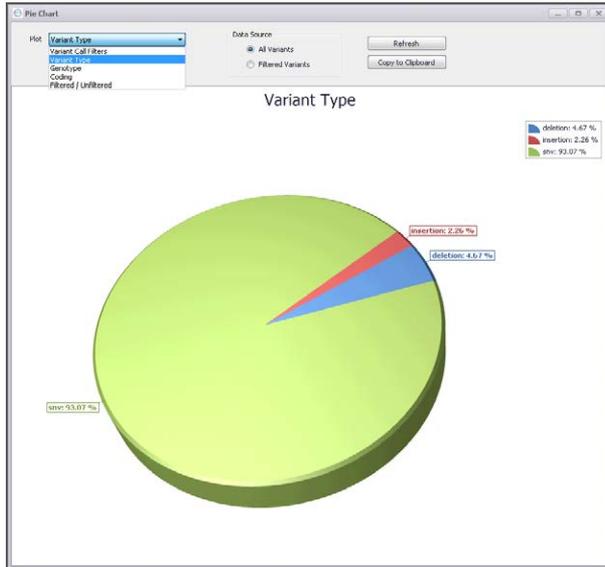


Figure 18 Pie Chart



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Technical Assistance

For technical assistance, contact Illumina Technical Support.

Table 1 Illumina General Contact Information

Website	www.illumina.com
Email	techsupport@illumina.com

Table 2 Illumina Customer Support Telephone Numbers

Region	Contact Number	Region	Contact Number
North America	1.800.809.4566	Japan	0800.111.5011
Australia	1.800.775.688	Netherlands	0800.0223859
Austria	0800.296575	New Zealand	0800.451.650
Belgium	0800.81102	Norway	800.16836
China	400.635.9898	Singapore	1.800.579.2745
Denmark	80882346	Spain	900.812168
Finland	0800.918363	Sweden	020790181
France	0800.911850	Switzerland	0800.563118
Germany	0800.180.8994	Taiwan	00806651752
Hong Kong	800960230	United Kingdom	0800.917.0041
Ireland	1.800.812949	Other countries	+44.1799.534000
Italy	800.874909		

Safety data sheets (SDSs)—Available on the Illumina website at support.illumina.com/sds.html.

Product documentation—Available for download in PDF from the Illumina website. Go to support.illumina.com, select a product, then select **Documentation & Literature**.

