Getting Started Guide

How do I log in and enter a license key?

- From the VarSeq welcome page, click on the Register tab and fill in the required information, read and accept the license agreement (EULA) and click **Register & Log In**.
- Once you are logged in, if you have not already activated a license key, or if you have reached the limit of activations available, you will be in VarSeq viewer mode.
- To activate a license key, click on **Activate License Key** in the lower right hand corner of the welcome page, or go to **Help > Activate a VarSeq License Key**. Enter in the provided license key and click **Verify**. If there are any available activations, click **Activate**.

How do I start a project?

- Click on a sample project shipped with VarSeq. They will be listed on the VarSeq welcome page or in **File > Open Example Project**.
- To create a new project, click on **Create New Project**.
- Select a project template from the list of existing templates. Information about each template will be displayed on the right.
- Verify the Genome Assembly matches the assembly used to align your data and specify a project name and location to save your project.
- An empty template can be used to create a new workflow. This is an advanced option.
- To open an existing project, either click on **Open Existing Project**, or select the project from the list of recent projects.

How do I add data?

- You can import one or more variant files into a new project. VarSeq also supports annotating from numerous other file types.
- If your project was created from a template, the Import Variants dialog will automatically appear after creating a new project. Otherwise, you can click on the **Import Variants** button and select one or more files to import.
- Once the files have been selected, compressed and indexed you will be prompted to edit the sample information. You can change the sample name, affection status, and if the samples are related, set the parents for the children. Sample information can be imported from a text file. BAM files can be linked to sample data for coverage statistics.
- Once the variants have been imported you may annotate the variants using annotation sources, or run a computation algorithm on the variants using sources already added.

How do I annotate my variants using existing sources?

- From the main tool bar, click on the ⊞Add button and select **Annotation…**. Choose the annotation source and then click **Select.** All imported variants will be annotated against the selected source.

How do I run an algorithm on my data?

- From the main tool bar, click on the ⊞Add button and select **Computed Data…**. On the left is a list of possible computation algorithms. Select the desired algorithm and click **OK**. Follow the prompts to add in the required parameters for the algorithm.

How do I change the samples visible or how the sample data is collated in a table?

- To change the samples visible or how the sample data is collated in a table, click on the sample display options icon ◇ and adjust either the **Collated By** or the visibility option for the samples.
- If the samples were imported with family information or tumor/normal pairs you have the option of only showing the current sample and linked samples (**Current and Parents or Tumor and Normal**). Otherwise these option are not available.

How do I change which columns or fields are visible in a table?

- Click on the Hide/Show icon. 👁 Entire groups of columns fields can be hidden or shown by toggling the check boxes.
- Expand each group to select particular fields to hide or show.
- Click on **Select All** or **Unselect All** either by a column group name or below all of the column groups to toggle the selection of more than one field at a time.

How do I sort data in a column?

- To sort data in a column, right-click on the column header and select one of the sorting options.
- To unsort the data, right-click on any column and select **Sort in Genomic Order**.

How can I visualize data in a particular filter?

- Click on the number of variants on the Filter Card and the table view will show the results of that step in the filter chain.
- To view the variants as they pass through each filter card, click on the **Expand** button on a particular card. The highlighted categories generally display the number of variants that pass the criteria specified by the card. Clicking on any of the numbers in the filter card will display those variants in the table view.
- Right-click on any filter card and select either **Results Up Through This Filter** or **Results For Only This Filter**.


The difference between result display options

Results Up Through This Filter

Selecting this option displays all variants that remain in the Filter Chain after applying the current filter. This means the particular filter criteria and **all** previous filter criteria have been met for the variants displayed in the Table View.

Results For Only This Filter

Selecting this option displays all variants from the original set of variants that meet the filter criteria of **only** the current filter.

How do I export data?

- Go to **File > Export** or click on the export button and select the format to export the data to.
- To export the data as a VCF file, you will need to select the fields in the table to export. By default only the fields visible in the table will be selected for export. Additional fields can be added by selecting them in the list of fields to export.
- To export the data to an Excel workbook, specify a file name. All selected tables will be exported into one workbook with one tab/sheet per table.
- Data can also be exported to a delimited text file or as an annotation source (TSF file).

NOTE: Only the variants present in the table(s) will be exported, respecting any applied filters.

How do I change the sample in the project?

- To quickly reprocess the filter chain for new samples, you can use the arrow keys on either side of the current sample menu to switch samples.
- To navigate through all samples in the project, click on the down arrow or right click in the current sample menu and select **Show All** then click on the right or left arrows or click on the down arrow and select a sample.
- To change the current sample, select the sample from the drop down menu.

How do I add a filter card to the filter chain?

- To add a filter card for a particular column or field in a table, right-click on the column and select **Create Filter Card for this Column**.
- To add a filter card to an existing filter container, right-click on the column to create a card and click on the created filter card and drag and drop the card into the desired container or position.
- You can also add a filter card or filter container by right clicking anywhere in the filter chain or on an existing container and selecting either **Add Filter** or **Add Filter Container**.

How do I mark variants in the table view?

- First create a record set by clicking on the **Record Sets** button 💬 and then click on the box for the particular variant you want to mark.

- To mark all current variants in a table select **Set to Current Variants** in the record set's sub-menu.
- To add all current variants in a table to an existing record set select **Add Current Variants** in the record set's sub-menu.

The difference between cards and containers

Filter Card
A filter card contains instructions on which variants to keep based on criteria specific to a single column or field of data.

Filter Container
A filter container can contain one or more filter cards and should be used for grouping filter cards together to create advanced criteria for determining which variants should pass the filter criteria.

How do I hide or stash a view?
- Click on any gray **X** in the corner of any tab. This will delete the view but it can be restored at any point with the existing settings.
- To stash a view, hover over one of the four corners of the window until the stash icon appears, for instance the icon in the upper left corner is: ⌐. Click on the icon and the tabbed view(s) closest to that corner will be stashed.

How do I rearrange the layout of tabs?
- Tabs (or views) can be rearranged like tabs in modern web browsers. Just click on a tab and drag and drop on an existing view to stack the tab, or hover over an edge of the window. If a placement hint appears, the tab can be dropped in that location.
- You can take advantage of multiple monitors by ripping tabs out of the main window and creating new windows. Additional tabs or views can be added to the new windows as well.

How do I add a new view or tab?
- Click on any plus sign next to a tab and select the type of tab to add to the stack of tabs at that current location.

- To add a new tab to one of the four corners of a VarSeq window, right-click on a stash icon and select the type of tab to add.

How do I visualize data in GenomeBrowse?
- To plot the variants, click on the plot button in the main tool bar and select **Plot Unfiltered Variants**.
- Similarly data from any numeric column and Sample Alignments from BAM files (if linked on import) can be visualized in GenomeBrowse through the plot menu on the main tool bar.

How do I create my own annotation source?
- In a GenomeBrowse view, click on the Add data sources button and in the lower left corner click on **Convert…**.

How do I edit labels?
- Double-click on any filter card, filter container, filter chain, plot title or axis label to edit the text.

How do I share a VarSeq project with a colleague?
- Anyone who has installed VarSeq can view existing projects. Have your colleague install VarSeq, and send them the entire project folder.
- They will be able to download any required annotation sources if they are publically available.
- Send with the project any proprietary annotation sources, or sources created locally.
- VarSeq Viewer allows free access to view projects, explore the filter chain and export results. Limitations include:
    - New projects cannot be created
    - New annotations cannot be added
    - Existing projects cannot be saved
    - Databases cannot be created or modified
    - Many filter chain modifications are disabled

How do I take notes or save important information about a project?
- You can either create a project note or connect to an Evernote account. To create a project note, add a **Note** view and select **Open a New Note**.

- To connect to an Evernote, create an Evernote account if you do not already have one. You can find a quick link to create an account in the File menu.
- Once you have an Evernote account, you will need to authorize VarSeq to have access to your Evernote account.
- After that step, you can either select an existing note or open a new note. This will open a new window that can be docked where ever you would like in any open window for the project.
- Notes can be saved, copied or shared with other users. Links to sites in GenomeBrowse can be added to notes to zoom to that location.

Using GenomeBrowse in VarSeq

How do I add a plot?

- You can drag and drop any BAM, BED, TSF or VCF file directly into the GenomeBrowse view. Additionally, if a BED or VCF file is bgzipped you can drag and drop the BED.GZ or VCF.GZ file into the view as well.
- You can also stream BAM and TSF files by clicking on the Add button in the top left corner of the GenomeBrowse view. From this dialog you can add data from a local folder, the Golden Helix public annotations server, or BAM files from a URL.
- You can change the default visible plots for Read Alignment (BAM) files by clicking on the GenomeBrowse view gear icon and checking or unchecking the **Coverage** or **Pile-up** options.
- You can change where new plots are added in the **Options** dialog. By default new plots are added to the **Top** of the plot view, this can be changed to have plots added to the **Bottom** of the view.

How do I navigate?

- There are two navigation modes in GenomeBrowse:
  - In Navigation Pointer Mode , you can pan any plot or axis by holding down the left mouse button. Dragging with the right mouse button scales the plot.
  - Zoom Mode will give you access to the rubber band zoom feature.

- You can use the scroll wheel to zoom in and out in any mode.
- By default, all plots have y-axis fit-data or auto zoom enabled. To change the y-axis zoom mode, hover over a plot and click the zoom mode button on the right edge (labeled with a letter).
- You can jump to a specific region or gene by typing it into the Zoom Toolbar. This includes typing in a gene or transcript name.
- There is a zoom slider for the x-axis in the toolbar between the – and + buttons. These controls scale the x-axis on all plots.
- There are left and right arrows framing the Domain Scale. These controls pan the x-axis on all plots.
- Right-click before you complete a zoom selection to cancel.

How do I see my previous view?

- Clicking the Back and Forward arrows   allows you to traverse zoom history.
- Right-clicking or holding the mouse button down on one of the buttons for a few seconds gives you a list of recent zoom history states, so that you can jump back by multiple steps.

How do I move and resize plots?

- You can drag plots around in the view using their handles (at the left of a plot).
- To resize the plot, you can mouse over the handle (at the bottom of a plot) and drag up or down.

How do I change how my data is displayed in a plot?

- In some plots, a gear icon will appear in the top-left corner when view options are available. Click on this gear icon to view plot-type specific options.
- If a view already contains the plot control panel, you just need to select a plot to have the control options update with those relevant to that plot type.
- To open the control panel, right-click on a plot and choose **Controls…**.

How do I change the colors?

- Click on the gear icon ⚙ and click on the **Color** tab.
- **Reference Mismatch** defines the color of variant plots when zoomed out.

What can I do with the mouse anchor?

- Right-click anywhere in a plot and choose **Place Mouse Anchor** to mark a location.

Mouse

Double-click on a title, scale, or filter card label to edit it in-place

Use the scroll wheel to navigate up or down in the view

Clicking on filter cards, filter containers, column groups, and columns, as well as rows in the Table View and features in the GenomeBrowse view will display details and statistics in the Detail View

In the Table View:
Use the arrow keys to select variants and view in GenomeBrowse and in the Detail View

Right-click on a row in the table view options for copying data from the row

Left-click on a column to display a histogram for the column

Double-click on a hyperlink to launch the web browser for that URL

In GenomeBrowse:
Use the scroll wheel to zoom in or out
- On canvas – affects x-axis only unless auto-zoom is on or a modifier key is used
- On scale – affects the scale axis only unless auto-zoom is on or a modifier key is used

Note for Mac OSX Keyboard Shortcuts
- Use the Command key instead of Ctrl
- Use the Option key instead of Alt

How do I learn more about the GenomeBrowse features?

- For more help consult the VarSeq manual or visit www.goldenhelix.com/GenomeBrowse/online_help

Keys

Alt + [Key]	Menu access
Ctrl + J	Show download window
F1	VarSeq Manual
Alt + 5	Show all stashed views
Alt + 1	Stash/unstash upper left corner
Alt + 2	Stash/unstash upper right corner
Alt + 3	Stash/unstash lower left corner
Alt + 4	Stash/unstash lower right corner
Ctrl + Alt + 1	Stash all but upper left corner
Ctrl + Alt + 2	Stash all but upper right corner
Ctrl + Alt + 3	Stash all but lower left corner
Ctrl + Alt + 4	Stash all but lower right corner
. (period)	Switch table view to filter chain output
Shift + .	Switch table view to filter chain input
Del	Delete selected filter cards or GenomeBrowse plots
Ctrl + C	Copy selected text
Ctrl + A	Select all (in any text box)
Ctrl + Z	Undo (in any text box)
Ctrl + N	New project
Ctrl + O	Open project
Ctrl + S	Save project
Ctrl + Shift + S	Save project as…
Ctrl + W	Close window
Alt + C	Cancel option dialog
Alt + O	OK option dialog

Golden Helix VarSEQ®

Original number of variants

Number of samples

Menu — File View Tools Help

Toolbar — Add   Export   💾 📂   ◄ Proband (NA19240) ▼ ► ⚙ ?

Samples: 3
Variants: 2,828,054

Stash Button

Stash Button

Trio Workflow ✕ +
☑ 🔒 Trio Workflow   🔧 2,828,054

☐ de Novo   🔧 ▮   ☑ ☐   ☑ ☐   ☑ Recessive Homozygous
  ☑ Read Depths (DP) (Current   🔧 □
    215,225   ☑ Read Depths (DP) (Cu
  ☑ Genotype Qualities (GQ) (C   🔧 □   ☑ Genotype Qualities (GC
    204,772
  ☑ All MAF < 0.01 OR missing   🔧 □   ☑ All MAF < 0.01 OR miss
    142,675
  ☑ Effect (Combined) is (LoF,   🔧 □   ☑ Effect (Combined) is Lo
    5,516   LoF
  ☑ de Novo Candidate   🔧 □   Missense
    111   Other
  ☑ ❗ NOT(Accession is miss   🔧 □   Missing
    3

Dominant Heterozygous   Compound Heterozygous

☑ Recessive Inheritance

Filter View

Selected Filter Card

Inverted Filter Card

Expanded Filter Card

Remaining Variants

3   170   400
403

GenomeBrowse ✕ +
Add   📷 ⚙   ◄ ► 18 ▼ 18: 48,604,672 - 48,604,679 ► ⇥
↖ 🔍 Homo sapiens (Human), GRCh37 g1k (Feb 2009) ▼ 👁 — ▮ +

18
18: 48,604,673   18: 48,604,675   18: 48,604,677   18: 48,604,679
◄   ►

Reference Sequence GRCH37 g1k, 1000Genomes   User Annotatio...
T G C A T A C

NA19240_modified   TRIOs
Pile-up
40
20
0
-20
-40

Variants - YRI_exome_trio_modified   Project Data
NA19238   ?
NA19239   ?-(?
NA19240   G

RefSeq Genes 105, NCBI   User Annotatio...

GenomeBrowse View

1 Variants) de Novo Can ✕   Genes) de Novo Can ✕ +
Variants ▼ 📊 👁 ◇ ▢ ► de Novo Candidate: NA192 ► Variants: 111

*Interesting Findings ✕ +
Interesting Findings ▼   📷 🔖 📷 📷 Tᴛ »

Detail ✕ +
☐ History   Copy   Clear

Table reflects last clicked filter output

	Variant Sites				Proband...	Mother ...	Fat ^
	Chr:Pos	Ref/Alt	Identifier	PV	Zygosity	Zygosity	
	17:14140081	G/A	rs1165...	☐	Heterozygous	Reference	
	17:27424239	A/C	?	▮	Heterozygous	Reference	
	17:39340848	G/C	rs7904...	▮	Heterozygous	Reference	
	17:46608203	A/G	rs1295...	☐	Heterozygous	Reference	
	17:59946430	C/G	?	☐	Heterozygous	Reference	
	18:14543039	T/C	rs4557...	☐	Heterozygous	Reference	
	18:14543063	A/C	rs4562...	☐	Heterozygous	Reference	
>	18:48604676	A/G	?	▮	Heterozygous	?	
	19:9085951	G/T	rs1118...	☐	Heterozygous	Reference	
	19:22156850	A/G	rs1998...	☐	Heterozygous	Reference	
	19:49657582	T/C	rs1140...	☐	Heterozygous	Reference	
	19:54724443	G/A	rs2017...	☐	Heterozygous	Reference	
	19:54745508	G/A	rs6173...	☐	Heterozygous	Reference	
	19:55790885	G/C	?	☐	Heterozygous	Reference	
	19:55790886	A/C	rs1998...	☐	Heterozygous	Reference	
	20:1895951	C/T	rs1496...	☐	Heterozygous	Reference	
	20:1895952	T/C	rs1461...	☐	Heterozygous	Reference	
	20:18464194	G/C	?	☐	Heterozygous	Reference	
	20:57767939	T/G	rs1847...	☐	Heterozygous	Reference	

Detail View describes selected variant

Interesting Findings

3: 162,829,126 - 162,831,716

Reference Sequence GRCH37 g1k, 1000Ge

NA19240_modified
Pile-up
2
-2

Variants - YRI_exome_trio_modified
NA19238
NA19239   G/T-(
NA19240

RefSeq Genes 105, NCBI
RPS6P4

18:48604676 - A/G

Variant Sites
Chr:Pos   18:48604676
Ref/Alt   A/G
Identifier ?
Show 23 hidden fields

Flags for Proband (NA19240)
PV True

Sample Fields Table
Samples	Zygosity
Proband (NA19240)	Heterozygous
Mother (NA19238)	?
Father (NA19239)	?
Show 8 hidden fields

NHLBI ESP6500SI-V2 Exomes Variant
Frequencies 0.0.19, GHI
All MAF ?

Navigation   (18: 32,506,870, 0.8125)   18   8 bp

Stash Button

Table View

Variant Set Flags

Note View

Detail View

Stash Button