User Guide

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# Table of Contents

Table of Contents ............................................................................................................. 2

**Background and Introduction** ......................................................................................... 4

  * Variant Calling Pipeline ................................................................................................. 4
  * Annotation Information Types ....................................................................................... 5

**Reports Page** ................................................................................................................... 6

  * Overview ......................................................................................................................... 6
  * Sharing Reports ............................................................................................................... 6
  * Viewing Reports .............................................................................................................. 6
  * Creating a New Report .................................................................................................... 7
    * Input Data ...................................................................................................................... 7
    * Summary Statistics ...................................................................................................... 7
    * Creating a Report ......................................................................................................... 8

**Variants Page** .................................................................................................................. 9

  * Overview ......................................................................................................................... 9
  * Title Bar ............................................................................................................................ 9
    1. iVariantGuide logo ....................................................................................................... 9
    2. Feedback, Help, Account Details ................................................................................ 9
    3. Report Title .................................................................................................................. 10
    4. Pathways and GO Terms Navigation Tabs ................................................................. 10
    5. Filters ........................................................................................................................... 10
      * Interactive Pie Charts ............................................................................................... 10
      * Interactive Histograms .............................................................................................. 11
      * Chromosome Region Selector ................................................................................... 11
      * Search Bar .................................................................................................................. 12
    6. Filter Presets .............................................................................................................. 12
Background and Introduction

iVariantGuide allows scientists to quickly and easily narrow thousands of variants down to a short list of candidate driver mutations. After upload to the web-based platform and a short processing step (usually less than 15 minutes), variants are automatically annotated with a rich variety of biological information. The interactive graphical filters allow users to identify patterns that would not otherwise emerge while rapidly identifying high-priority variants. Advaita provides an additional layer of biological context by allowing users to visualize their high-priority variants in the context of relevant pathways (including associated miRNAs and drugs) and gene ontology terms.

Variant Calling Pipeline

Before Variant Analysis there are several key steps to processing Next-Gen Sequencing (NGS) data. The sequencer performs primary analysis by interpreting the sample data to infer a genotype for every nucleotide. Next, the reads generated during sequencing are aligned to generate a single consensus sequence. The final step before variant analysis is variant calling. During this secondary analysis step, the consensus sequence is compared to a reference assembly to identify mutations, i.e. genotypes that are different from the reference sequence. These so-called variants are recorded in a variant call file (VCF), including information about chromosome, position, reference allele, alternate allele and metrics for call quality and read depth. This variant call file (VCF) is used as the input to iVariantGuide.
Annotation Information Types

Variants are annotated with a variety of types of information. These annotations can subsequently be used to filter and prioritize variants to identify driver mutations, characterize novel variants, and more.
Reports Page

Overview

The Reports Page is the main landing page. It contains all reports that you have created or that have been shared with you. This is where you can create new reports and share and analyze reports you create.

There are three possible status indications for each report:

- **CREATED**: a report that is currently being processed
- **ERROR**: a report that has failed processing
- **PURCHASED**: a report that was successfully processed (and you may access)

Sharing Reports

If the share icon in the report bar is dark grey, you have sharing permissions for that report. Click the share icon to open the sharing dialogue. You may enable a public link to share with anyone or enter an individual email address to send a private link.

Viewing Reports

You will automatically receive an email as soon as your analysis is complete. When the status bar is green, your report is ready to start exploring! Click the link icon to be taken directly to your report. Clicking the reports bar and selecting EXPLORE will also allow you to enter the report.

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Creating a New Report

The icon on the right hand side of the Reports page takes you to the Report Creation page. Fill in the Report Details in the right hand panel, including Title and Description. Select a sample to be analyzed from those available in the left hand panel, or upload a new sample as a VCF file.

To upload a new sample, click the blue circle above Available samples.

Input Data

iVariantGuide accepts variant call files meeting the following criteria:

- **Type**: .vcf OR .vcf.gz
- **Size**: .vcf less than 100MB, vcf.gz under 20MB
- **Version**: .vcf 4.1 or later
- **Assembly**: reference genomes hg19 (GRCh37) or GRCh38

Summary Statistics
Once your data is uploaded, you may view File stats. When you are satisfied, click SAVE SAMPLES to add it to your Available samples. If ever you want to revisit these summary statistics, you can view them by selecting either the sample or the report, and clicking the bar chart icon next to File stats.

**Creating a Report**

With title, description, and sample fields all complete, you may now create the new report by clicking **ANALYZE DATA**. The report status is **PENDING** until your data analysis is complete.
Variants Page

Overview

The variants page contains information about the variants in your file. This is where you can view statistics, apply filters and presets, and view details about individual variants. Each of the numbered features in the screenshot are described in detail below.

Title Bar

1. iVariantGuide logo

Clicking the logo returns you to the reports page from any screen.

2. Feedback, Help, Account Details

The chat bubble icon provides a way for you to submit feedback to the Advaita development team. In the dialogue that pops up, please select whether your feedback is an issue, feature request, or other/ question. Then tell us a bit about your issue or idea. We’ll get back to you as fast as we can!
The circled question mark opens a new tab in your browser and takes you to the Support section of the iVariantGuide website. There, you will find tutorials in the form of videos, webinars, FAQs, and this User Manual.

The silhouette indicates where you can change your password, access the EULA and Privacy Policy, or log out of your account.

3. Report Title
This bar tells you which report you are exploring using the title you entered when you created it and the time it was created. If a preset is selected, it will be noted in parentheses and can be cleared by clicking the X.

4. Pathways and GO Terms Navigation Tabs
These tabs allow you to navigate between the Variants, Pathways, and GO Terms pages. The current page is indicated by blue font with a green underline.

5. Filters
The graphical filters in iVariantGuide provide an interactive view of the filtered variant set. Clicking on any of the graphs allows users to select and deselect filters. At the moment any filter is changed all other graphs update dynamically. Every graphical filter may be exported as a .png or .svg image. In the open view of the filter, click the download arrow in the upper right corner and select the file type in the dialogue that opens.

Interactive Pie Charts
Many of the filters can be displayed as pie charts representing the breakdown of sub-filters in the variant set. To view/ hide a pie chart, click on the pie icon on the left side of its associated title bar. When a pie chart is open, sub-filters may be selected by clicking directly on the associated slice of pie. When a single sub-filter is selected, clicking on the pie chart deselects the filter, causing all sub-filters be included. Sub-filters may also be selected and deselected by clicking the title bar and checking or unchecking boxes in the dropdown menu that appears.
Interactive Histograms

For filters described by numeric thresholds rather than discrete categories, the summary information is given by a histogram. To set a threshold, hover over the desired threshold value and click. To clear the threshold, click the red circle in the upper right corner of the shaded region.

Chromosome Region Selector

When a single chromosome is selected in the Chromosome filter, users may further narrow the selection by highlighting a region of that chromosome. To adjust the boundaries of the highlighted region, users may drag either edge to the desired position. Clicking and holding in the center of the highlighted region allows users to slide the entire window along the chromosome map.
**Search Bar**

The search bar offers yet another way to filter variants. Terms entered here cause the variant set to be filtered according to associated diseases and/or impacted genes. Typing multiple terms includes all variants matching any term (OR search). Search terms can be saved as presets, alone or in combination with any other filters.

**6. Filter Presets**

Presets are combinations of filters that users may save and apply to any report. In addition to the convenience of applying multiple filters simultaneously, selecting a preset preserves the resulting set of filtered variants for analysis in the Pathways and GO Terms pages.

The list of presets you have previously saved can be opened or hidden from the left side view. Search within your presets using the search bar above the preset list. To view the filter combination represented by any preset, click the info icon to the right of its name.

**Applying Presets**

Clicking a preset name in the list selects that preset and applies all filter settings specified therein. At the moment the preset is applied, all other filters are automatically cleared. When a preset is applied, its title appears in the light blue title bar.

**Saving Presets**

Presets may include any of the interactive filters as well as search terms on the variants page. To save a new preset, apply the desired filter settings then click **SAVE AS** in the light blue title bar. The preset is not saved until you enter a name in the dialogue window that opens and click **SAVE**.

If you do not save your filters as a preset before navigating to the Pathways or GO Terms pages, the filters will not be applied to the variants represented on those pages. If you navigate away from the Variants Page without saving the current filter settings, a warning message will appear...
in the light blue bar. Clicking the red warning symbol takes you back to the variants page where you can save the current settings before continuing your analysis. The same warning will appear if, after selecting a preset, you change one or more filter settings and then navigate away from the variants page without saving your new selection.

**Clearing Presets**

After a preset is selected, it may be cleared by clicking the X next to its name in the light blue title bar.

**7. Variants Table**

The Variants Table lists all variants remaining in the filtered set. By default, the table is sorted by chromosome and position, and clicking the arrow next to the column header reverses the sort order. The table may be exported in .csv format by clicking the download icon in the upper right corner. At the bottom right of the table, the total number of filtered variants is shown (circled).

**Variant Details Page**

Clicking on any entry in the table opens the Variant Details page from the right. This page gives detailed information about a single selected variant.

**dbSNP**

At the top of the Variant Details page, the dbSNP identifier associated with the variant and a link to its dbSNP entry are given.

**Transcripts/Protein change**

This table lists all annotations for this mutation. Changes occurring in multiple transcripts are listed separately, as well as alternate annotations of the same allelic change in a single transcript.
**Diseases**

Diseases associated directly with the variant, as documented in ClinVar, are listed with a single entry for every ClinVar accession ID. When available, links to more information about the disease from GeneReviews, MedGen, OMIM, Orphanet, and SNOMED are also provided.

**Pathways**

All pathways containing genes impacted by the selected variant are listed, ranked by the number of genes contained in each that are impacted by variants in the filtered set. Clicking on any pathway takes you to the Pathway Details view for that pathway.

**GO Terms**

All Gene Ontology Terms associated with genes impacted by the selected variant are listed, ranked by the number of genes associated with each term that are impacted by variants in the filtered set. Clicking on any GO Term takes you to the GO Term Details view for that pathway.
Pathways

The Pathways page allows you to explore the biological context of your filtered variant set. To maintain the filter settings from the Variants page, a preset must be selected. If there is no preset corresponding to the current filter settings, click the green **SAVE AS** button at the top of the page, and name a new preset before navigating to the Pathways page.

The Pathways page is reached in one of two ways: 1) by selecting the PATHWAYS tab beneath the title bar; or 2) by clicking a pathway name in the Variant Details view.

Pathways List

All pathways that contain genes associated with the filtered variant set are listed. The pathways are ranked by the number of impacted genes they contain and the predicted level of impact. To reverse the sort order, click the arrow next to Pathways in the header.

Pathway Details

Clicking any pathway in the list opens the pathway details view on the right. Pathway nodes with genes associated to variants in the filtered set are highlighted according to the predicted level of impact.

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Hovering over a highlighted node reveals the list of impacted genes for that node. Each gene is selectable, which causes the variant set to be filtered to only those associated with that gene.

**Associated Variants**

Variants associated with genes on the pathway (or selected genes from the pathway, if any are selected) are listed below the pathway map. Clicking a variant in this table navigates away from the Pathways page to the Variant Details page.

![Variant Table](image)

**miRNAs**

miRNAs associated with any genes on the pathway (if none are selected) or selected genes are listed next. Selecting a miRNA updates the pathway map with teal ovals indicating genes that are targets of the selected miRNA.

**Drugs**

Similar to miRNAs, drugs that are known to target selected genes or any genes on the pathway if none are selected are listed. Selecting a drug traduces orange ovals to the pathway map indicating which genes are targeted by the selected drug.
References

References from PubMed are listed below. Links are included to view the publication in PubMed.
GO Terms

The GO Terms page allows you to explore the biological context of your filtered variant set. To maintain the filter settings from the Variants page, a preset must be selected. If there is no preset corresponding to the current filter settings, click **SAVE PRESET** and name a new preset before navigating to the GO Terms page.

The GO Terms page is reached in one of two ways: 1) by selecting the GO TERMS tab beneath the title bar; or 2) by clicking a GO term in the Variant Details view.

GO Terms List

All GO terms that contain genes associated with the filtered variant set are listed. The pathways are ranked by the number of impacted genes they contain and the predicted level of impact. To reverse the sort order, click the arrow next to GO Terms in the header.

GO Term Details

Clicking any GO term in the list opens the pathway details view on the right. The selected term will be displayed in a tree with all of its ancestor terms. Each term contains a color bar representing the number of genes in the filtered variant set associated to that term that are predicted to have high, moderate, low, and modifier impact. The GO tree can be moved by clicking and dragging in the window. Selecting
“Show legend” reveals a legend that defines arrow colors for interaction types. Clicking the GO number at the top of any GO term changes the selected term to that one.

**Associated Variants**

Variants predicted to impact genes associated with the GO term are listed below the Ancestor chart. Clicking a variant in this table navigates away from the GO Terms page to the Variant Details page.

**Parent and Child GO Terms**

Below the Variant list, the parents and children of the selected GO term are listed. As in the overall GO Terms list, they are sorted by the number of high, moderate, low, and modifier impacted genes. Clicking a GO term from either list updates the ancestor tree to be based around the selected GO term.

**References**

References from PubMed are listed below. Links are included to view each publication in PubMed.
Citing iVariantGuide

Using Advaita Bioinformatics’ products or content for any form of publication (e.g. print, electronically) requires researchers to cite them. Please use one of the options below for citations:

• “The Data (significant variants, associated genes, predicted impacts, clinical significance, impacted pathways, GO terms, etc.) were analyzed using Advaita Bioinformatics’ iVariantGuide (http://www.advaitabio.com/ivariantguide)”.

• LaTeX users may use the following code in the bibtex file:

```latex
~\cite{advaita2016}

@ONLINE{advaita2016,
  author = {Advaita, Corporation},
  title = {Variant Analysis with iVariantGuide},
  month = May,
  year = {2016},
  url = {http://www.advaitabio.com/ivariantguide.html}
}
```

For additional guidance, please contact info@advaitacorporation.com.