



User Guide

v. 0.9

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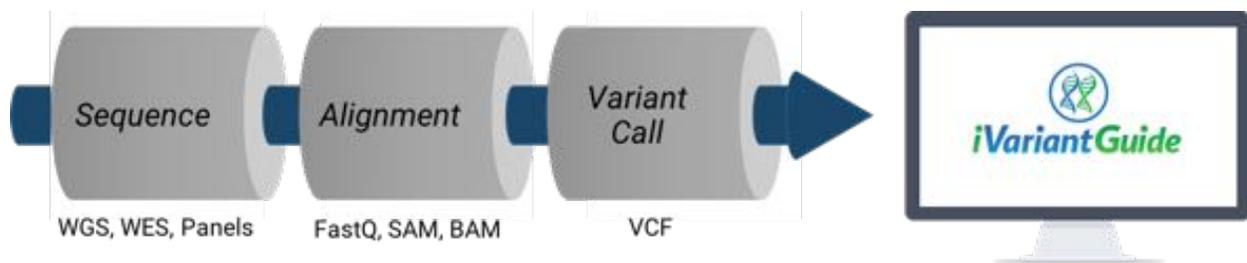
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Background and Introduction

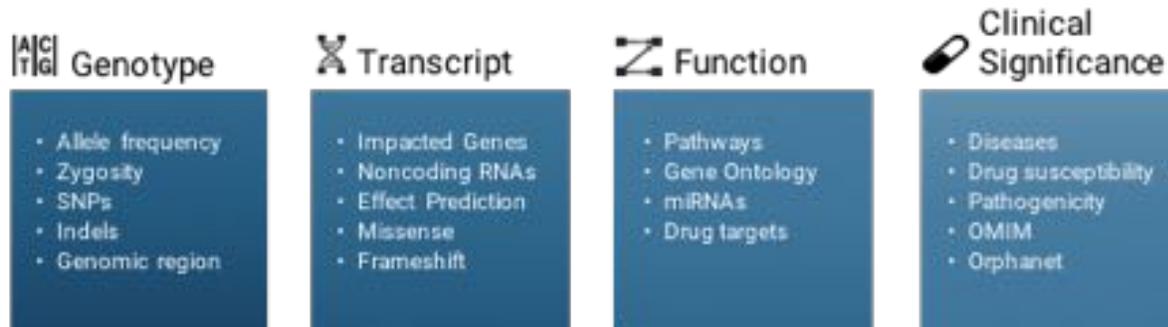
iVariantGuide allows scientists to quickly and easily narrow thousands of variants down to a short list of candidate driver mutations. After upload to the web-based platform and a short processing step (usually less than 15 minutes), variants are automatically annotated with a rich variety of biological 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.

Name	Type	Last updated	Status
PanCancer 280 - updated ClinVar	VARIANT_SINGLE_SAMPLE	2017-07-14 09:03 PM	PURCHASED
PanCancer 101 - updated ClinVar	VARIANT_SINGLE_SAMPLE	2017-07-14 09:02 PM	PURCHASED
Genetic Variation	VARIANT_SINGLE_SAMPLE	2017-07-14 08:04 PM	ERROR
Genetic Variation	VARIANT_SINGLE_SAMPLE	2017-07-14 08:04 PM	ERROR
Genetic Variation	VARIANT_SINGLE_SAMPLE	2017-07-14 08:04 PM	PURCHASED
Genetic Variation	VARIANT_SINGLE_SAMPLE	2017-07-14 11:03 AM	PURCHASED
Genetic Variation	VARIANT_SINGLE_SAMPLE	2017-07-14 11:03 AM	PURCHASED
Genetic Variation	VARIANT_SINGLE_SAMPLE	2017-07-14 10:01 AM	PURCHASED
Genetic Variation	VARIANT_SINGLE_SAMPLE	2017-07-14 10:01 AM	PURCHASED
Genetic Variation	VARIANT_SINGLE_SAMPLE	2017-07-14 10:01 AM	PURCHASED

There are three possible status indications for each report:

PENDING : 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

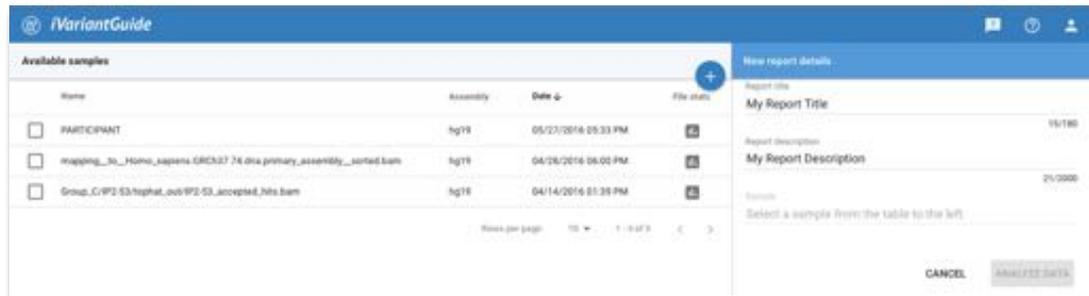
If the share icon in the report bar is dark grey, you have sharing permissions for that report. Click the share icon to open the sharing dialogue. You may enable a public link to share with anyone or enter an individual email address to send a private link.

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.

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**.

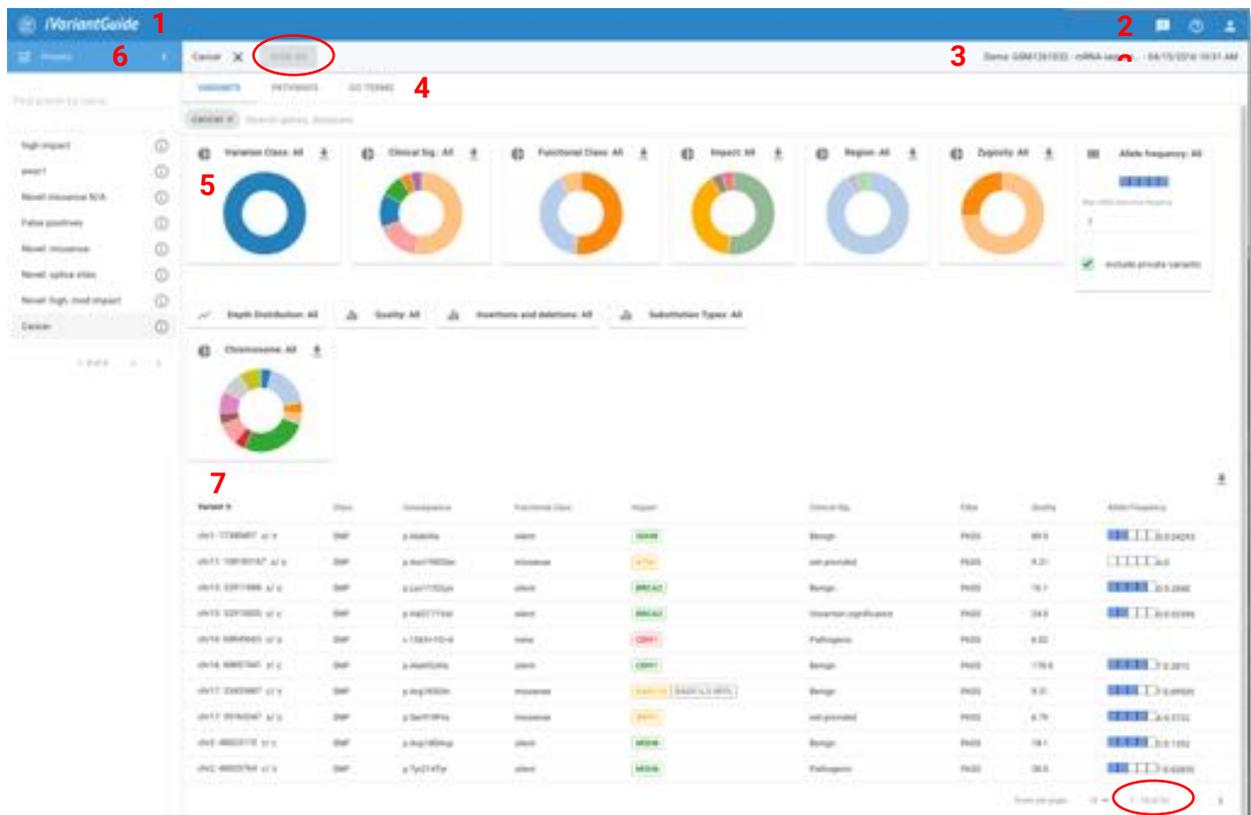
Creating a Report

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.

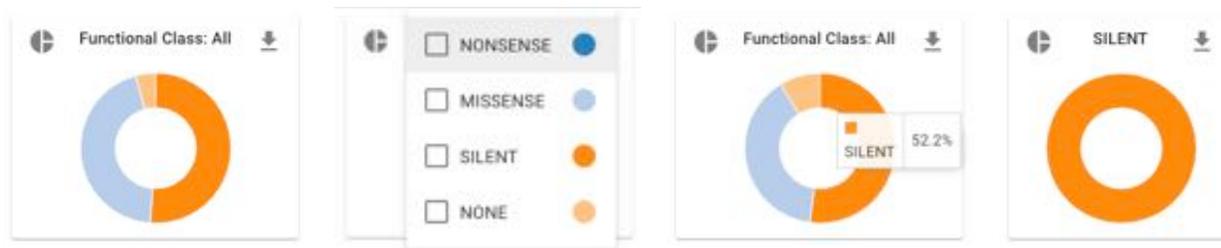
5. Filters

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.

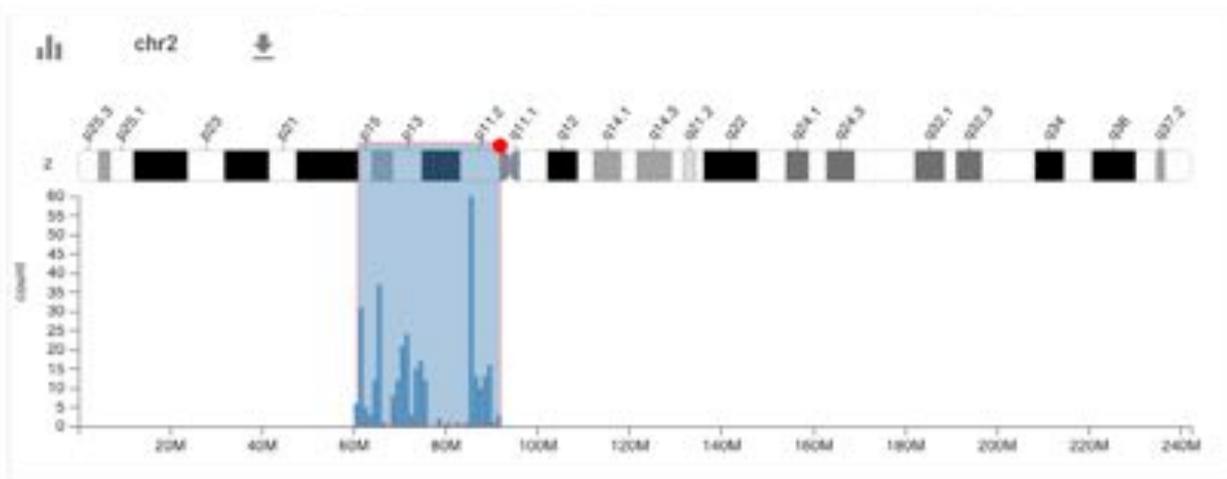
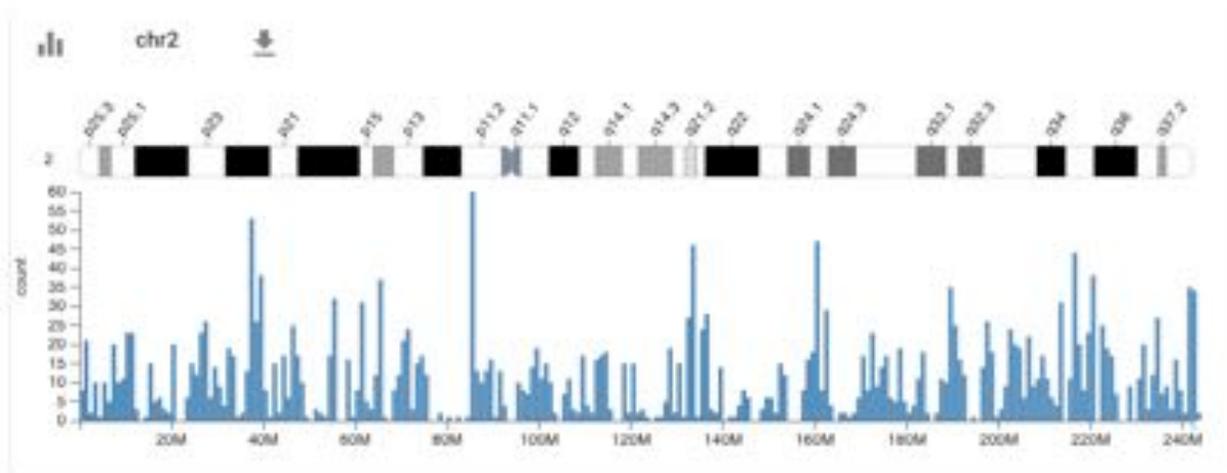


Interactive Histograms

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.

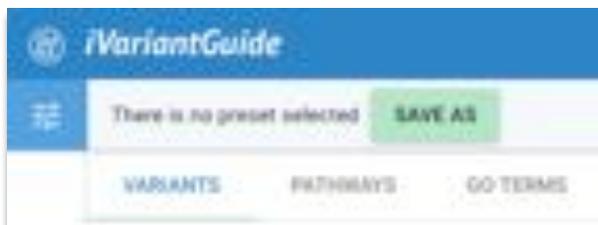
 The list of presets you have previously saved can be opened or hidden from the left side view. Search within your presets using the search bar above the preset list. To view the filter combination represented by any preset, click the info icon to the right of its name.

Applying Presets

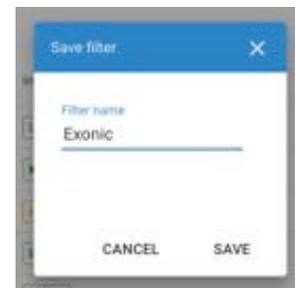
Clicking a preset name in the list selects that preset and applies all filter settings specified therein. At the moment the preset is applied, all other filters are automatically cleared. When a preset is applied, its title appears in the light blue title bar.

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**.



Variants Page without saving the current filter settings, a warning message will appear

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

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

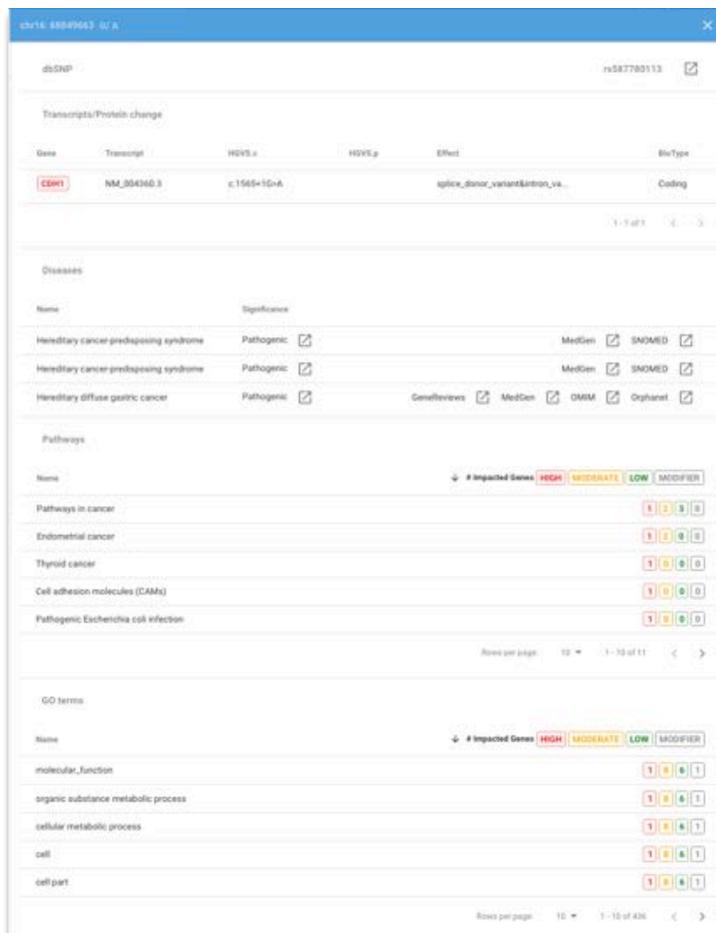
Clicking on any entry in the table opens the Variant Details page from the right. This page gives detailed information about a single selected variant.

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.



The screenshot displays the Variant Details page for variant rs87780113. The top section, 'Transcripts/Protein change', contains a table with the following data:

Gene	Transcript	HGVS	Effect	BiType
CDH1	NM_004063.3	c.1565+1G>A	splice_donor_variant&tron, va...	Coding

Below the table, there are sections for 'Diseases', 'Pathways', and 'GO terms'. Each section lists associated terms and their significance levels (HIGH, MODERATE, LOW, MODIFIER).

Diseases:

Name	Significance	MedGen	SNOMED
Hereditary cancer-predisposing syndrome	Pathogenic	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
Hereditary cancer-predisposing syndrome	Pathogenic	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
Hereditary diffuse gastric cancer	Pathogenic	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>

Pathways:

Name	# Impacted Genes	HIGH	MODERATE	LOW	MODIFIER
Pathways in cancer	1	1	0	0	0
Endometrial cancer	1	1	0	0	0
Thyroid cancer	1	1	0	0	0
Cell adhesion molecules (CAMs)	1	1	0	0	0
Pathogenic Escherichia coli infection	1	1	0	0	0

GO terms:

Name	# Impacted Genes	HIGH	MODERATE	LOW	MODIFIER
molecular_function	1	1	0	0	0
organic substance metabolic process	1	1	0	0	0
cellular metabolic process	1	1	0	0	0
cell	1	1	0	0	0
cell part	1	1	0	0	0

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.

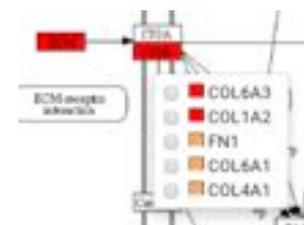
Pathways List

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.

The screenshot shows the VariantGuide interface. On the left, there's a 'Pathways List' with various categories and their corresponding gene counts. The main area on the right shows a detailed pathway diagram for 'Focal adhesion'. The diagram includes a description of the pathway's role in cell-matrix interactions and a network of nodes representing proteins and their interactions. Several nodes are highlighted in red, indicating they are impacted by the filtered variant set.

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.



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	Class	Gene	Significance
chr10:35211848 c/a	SNP	ITIH4	N/A
chr13:11843030 c/a	SNP	COL6A1	N/A
chr18:3253428 a/a	SNP	MYL12A	N/A
chr2:21423589 c/t	SNP	ITIH1	N/A
chr2:21423780 a/a	SNP	ITIH1	N/A
chr2:21430048 c/a	SNP	ITIH1	N/A
chr2:23403292 c/t	SNP	COL6A3	N/A
chr21:4741037 c/t	SNP	COL6A1	N/A
chr7:14024472 a/ cctctcttc	del	COL1A2	N/A
chr7:14025754 a/ aa	del	COL1A2	N/A

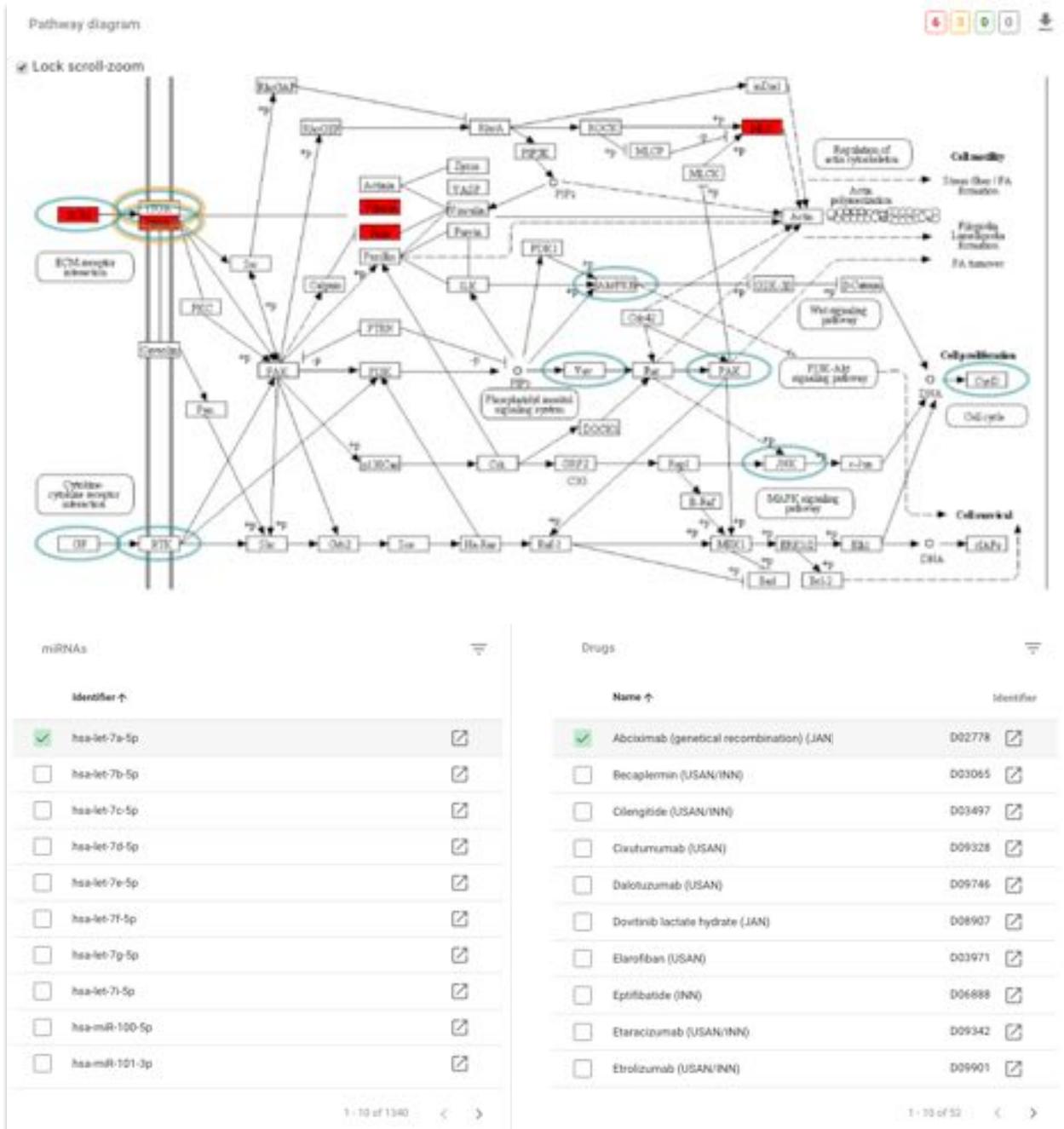
Items per page: 10 1 of 10

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.

References		
Authors ↑		
Berman AE, Kozlov N, Mizanovich SE. <i>Biochemistry (Mosc)</i> 68:1284-99 (2003) Integrins: structure and signaling.	PubMed	14758828
Burdige K, Wenzelberg R. <i>Cell</i> 118:157-70 (2004) Rho and Rac take center stage.	PubMed	14744429
Comoglio PM, Boccaccio C, Truscetti L. <i>Curr Opin Cell Biol</i> 15:585-91 (2003) Interactions between growth factor receptors and adhesion molecules: breaking the rules.	PubMed	14516385
Damen EH, Yamada KM. <i>J Cell Physiol</i> 189:1-13 (2001) Fibronectin, integrins, and growth control.	PubMed	11575199
Gao W, Giancotti FG. <i>Nat Rev Mol Cell Biol</i> 5:816-26 (2004) Integrin signaling during tumour progression.	PubMed	15439642
Habekova B, Krivkova J, Barak J, Lukac J, Keller Z. <i>Biomol Pap Med Fac Univ Palacky Olomouc Czech Repub</i> 147:19-25 (2003) The mechanism of action of the tumour suppressor gene PTEN.	PubMed	15034601
Lee JH, Juliano R. <i>Mol Cells</i> 17:189-202 (2004) Mitogenic signal transduction by integrin- and growth factor receptor-mediated pathways.	PubMed	15179230
Mitra DK, Hanson SA, Schlaepfer DD. <i>Nat Rev Mol Cell Biol</i> 6:54-68 (2005) Focal adhesion kinase: in command and control of cell motility.	PubMed	15499017
Nicholson KM, Anderson NG. <i>Cell Signal</i> 14:387-95 (2002) The protein kinase B/Akt signaling pathway in human malignancy.	PubMed	11882383
Okumi A, Miyaue T, Oeda H. <i>Biochim Biophys Acta</i> 1591:1-15 (2004) Regulation of E-cadherin expression and beta-catenin/Tcl transcriptional activity by the integrin-linked kinase.	PubMed	15053919

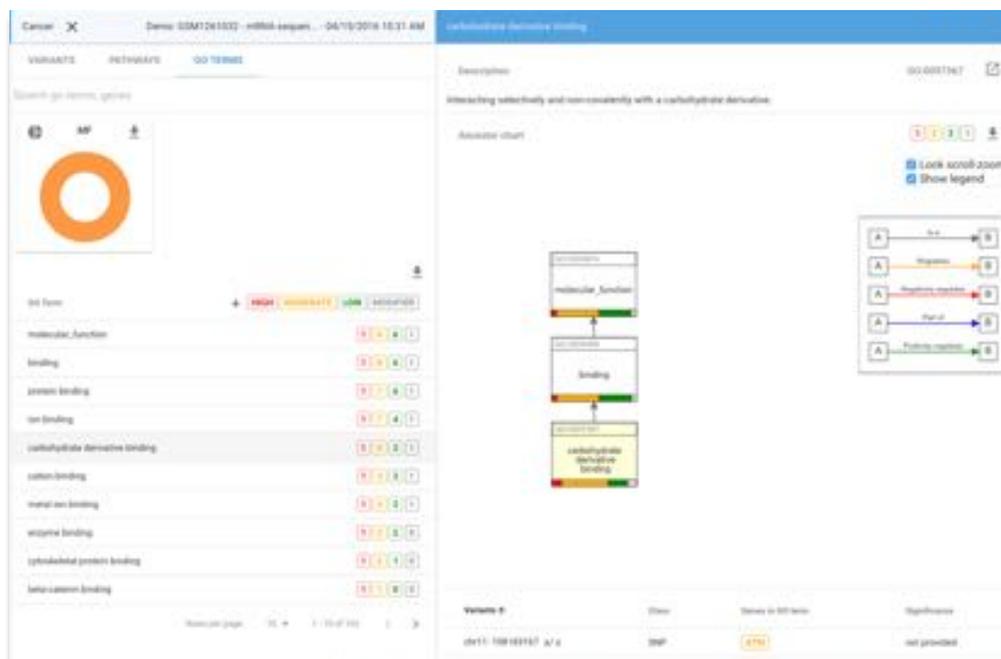
GO Terms

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:

```
~\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.