



# Ingenuity<sup>®</sup> Variant Analysis<sup>™</sup> Quick-Start Guide

For accessing and using Ingenuity Variant Analysis

Ingenuity® Variant Analysis™ is a web-based application that combines analytical tools and integrated genomics content with user panel, exome, or genome data to rapidly identify and prioritize compelling causal variants using published biological evidence. This Quick-Start Guide summarizes how to access the online application, upload data files, perform and share analyses.

Before using Variant Analysis, visit <https://www.qiagenbioinformatics.com/products/ingenuity-variant-analysis/> where you can access pre-recorded webinars, reference materials and profiles of featured researchers who have used the product. Tutorials are available at <http://ingenuity.force.com/variants/VariantTutorials>.

The “Help” function in Variant Analysis is a comprehensive, keyword-searchable resource, designed for quickly finding necessary information and instructions. Click “Help” at the top of the Variant Analysis application or visit <http://ingenuity.force.com/variants/VariantTutorials>.

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## Access Variant Analysis

Anyone with a valid email address\* can register for a trial account by going to the secure site <https://www.qiagenbioinformatics.com/products/ingenuity-variant-analysis/> and clicking “Request a trial”.

After registering, log into Variant Analysis at <https://variants.ingenuity.com/va/>, where you will find published datasets to explore and instructional videos to get started.

You can reset your username or password at <https://apps.ingenuity.com/isa/account/forgotpassword> or contact Customer Support at <http://www.ingenuity.com/customer-support> or +1 (650) 381-5111.

\*Company or institute email address

## Uploading data files to Variant Analysis

Variant Analysis is a web-based application delivered via the Ingenuity secure private cloud environment. User data to be analyzed must be uploaded by clicking “My Samples” in the top navigation bar.

Variant Analysis supports called variant files from human whole genome, whole exome, or targeted gene panel files that are in one of the following formats:

- Variant Call Format (VCF)
- Genome Variation Format (GVF)
- Complete Genomics files (Var, MasterVar, High confidence junction, etc.).

More information about file format requirements and uploading data can be found in Variant Analysis Help under “Upload your sample”. Samples can be annotated in Variant Analysis with pedigrees, clinical features, and sample pairings, with an individual subject ID. To learn how, see “Annotating your samples” in Help.

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VARIANT ANALYSIS

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[Create](#) [Refresh](#) [Share](#) [Open](#)

Name	Description	From	Created	Samples	Status	
Pre-Filtered Analysis		ryp@ingenuity.com	11/10/2015	1 / 2	active	▲

**User Settings** ✕

☒ Opt-in to share anonymized, pooled statistics derived from my samples and gain access to the [Allele Frequency Community database](#).

[Apply](#)

## Creating an analysis

Start by selecting the samples to analyze together. Variant Analysis will merge the variants into a single analysis and annotate all variants using available content from the Ingenuity Knowledge Base.

1. Click the "My Sample" tab in the top navigation bar, and then select the sample(s) you wish to include in your analysis.
2. Click the Analyze button to begin the Analysis build wizard

[Analyze](#)

- Confirm your case and control samples are properly grouped otherwise you can click and drag samples from one window to the other. When you are satisfied with your analysis design, click the Next button on the lower right to proceed.

**1 Cases and Controls** 2 Focus the Analysis 3 Sample-specific options 4 Analyze

Select and drag samples to designate case/control status.  
Drag samples from the left table to the desired list on the right. Drag to reorder samples. Their order here determines their order within the analysis views.

Search samples by keyword

Name	Subject	Created
Patient 46 adj NT	SRR1555248	09/22/15 04:25 PM
Patient 46 T	SRR1555095	09/22/15 04:25 PM
PG0001695-BLD	05-04	08/11/15 11:37 AM
PG0001697-BLD	06-02	08/11/15 11:37 AM
PG0001696-BLD	06-01	08/11/15 11:37 AM
PG0001690-BLD	04-05	08/11/15 11:37 AM
PG0001689-BLD	04-04	08/11/15 11:37 AM
PG0001691-BLD	04-01	08/11/15 11:37 AM
PG0003562-BLD	05-04	08/11/15 11:37 AM
PG0003573-BLD	05-02	08/11/15 11:37 AM
PG0003565-BLD	05-03	08/11/15 11:37 AM
PG0003524-BLD	05-01	08/11/15 11:37 AM
PG0001693-BLD	07-03	08/11/15 11:37 AM
PG0001692-BLD	07-02	08/11/15 11:37 AM
PG0002917-DNA	07-05	08/11/15 11:37 AM

3 Cases (affected, tumor, responder, etc.)

- PG0001707-BLD
- PG0001706-BLD
- PG0001705-BLD

3 Controls (unaffected, normal, nonresponder, etc.)

- PG0001698-BLD
- PG0001708-BLD
- PG0001704-BLD

Use a control library

Back Next

- Select your desired Analysis Design. Note that you can always alter your analysis settings even after the analysis has completed. Click Next in the bottom right to proceed.

**1 Cases and Controls** **2 Focus the Analysis** 3 Sample-specific options 4 Analyze

Please select the type of analysis you would like to start with.  
This will set your starting filters to best practices. You can always change these settings later. If multiple options apply, start with one and start another analysis with a different selection later.

**Analysis Design**

- ☒ Genetic disease  
Identify causal variants using trios, family analysis or case vs control models.
- ☐ Cancer  
Focus on somatic variants
- ☐ Stratification study  
Typically used to study complex disorders and with large numbers of samples (at least 50 recommended). Compare cases and controls statistically
- ☐ Other  
Use the most general filter settings.
- ☐ Settings from a previous analysis  
Pre-Filtered Analysis - 11/10/2015

Back Next

**1 Cases and Controls** **2 Focus the Analysis** 3 Sample-specific options 4 Analyze

Please select the inheritance model you are interested in.  
If you aren't sure, best practice is to start with de novo and explore the other options later as needed. Your selection will set the options in the Genetic Analysis filter which you can change at any time.

**Type of the inheritance for a trio analysis of a genetic disease:**

- ☐ Dominant
- ☐ Recessive - Compound het model (typically used when both parents are unaffected, and not both from the same founding population)
- ☐ Recessive - Homozygous model (typically used when at least one parent is affected, parents are consanguineous or are both from the same founding population)
- ☒ De novo  
Affected case/child is likely to have a de novo variant
- ☐ Not from the same family (not a trio)  
Treat as independent cases and controls

Back Next

- If your analysis consists of a trio of samples the Wizard will assume you would like to perform a trio analysis and will offer mode of inheritance analysis options. If your analysis is not a trio, the wizard will proceed straight to the Biological Context window where you can optionally specify any relevant biological term(s) to help narrow down the list of variants likely contributing to disease or phenotype. Variant Analysis will use this information to automatically focus initial results to a short list of relevant variants. The terms used in the analysis can be changed at any time.

**1 Cases and Controls** **2 Focus the Analysis** 3 Sample-specific options 4 Analyze

Optionally annotate and filter variants based on biological context  
Select something fairly broad (ie, the main disease name rather than a specific subtype.) Providing biological terms is highly recommended so that you can easily find relevant literature and citations. Adding these terms will not constrain your results. You can always view the variants that are not annotated biologically as well as those that are.

This dataset concerns: Genetic disease

Which biological terms describe this disease?

Enter relevant diseases, phenotypes, pathways, processes, or domains

cranios

- craniosclerosis [disease]
- craniosclerosis of coronal suture [disease]
- craniosclerosis of sutura lambdoidea [disease]
- autosomal dominant craniosclerosis [disease]
- autosomal recessive craniosclerosis (autosomal recessive craniosynostosis) [disease]

Back Next

6. This next Wizard window will only appear if you are analyzing genomes. You may choose to analyze only the exonic regions or entire regions of your genome(s). Note that due to compute resource requirements, Variant Analysis has a default upper limit of 299 genomes that are allowed full genome analysis. To perform full genome analysis of greater than 299 genomes, please contact Customer Support.

The screenshot shows the 'Considerations for whole-genome analysis' window, which is part of a four-step wizard. The window title is '1 Cases and Controls'. The main text reads: 'To make the size of the analysis you are creating manageable, Ingenuity recommends selecting at least one of these pre-filtering options. Pre-filtering will remove the least scientifically useful data so you can focus your analysis on regions of greater interest.' There are three checkboxes: 'Keep only variants in Exonic regions' (checked), 'Exclude common variants that are observed with high allele frequency in public databases' (unchecked), and 'Keep only variants above minimum confidence standards' (unchecked). Each checkbox has a 'show details' link next to it. At the bottom, there are 'Back' and 'Next' buttons.

7. If your VCF file contains annotations, you can optionally click and drag up to 5 annotations to be part of your analysis.

The screenshot shows the 'Your VCF data sets include variant annotations' window, which is part of a four-step wizard. The window title is '2 Focus the Analysis'. The main text reads: 'Your VCF data sets include variant annotations that you can include in your analysis for custom filtering. Select in-house annotations only since those from public databases are already included. Drag annotations from the left table to the list on the right as custom annotation columns. Drag to reorder annotations. Their order here determines their order within the variants view.' There are two tables: a left table with columns 'Name' and 'Description' containing 12 annotations (AC1, AF1, CLR, FQ, HWE, MQ, PCHI2, PR, QCHI2, VDB), and a right table titled '0 Annotations'. At the bottom, there are 'Back' and 'Next' buttons.

8. Finally, provide a name for your analysis and optionally a description. Click Analyze to begin your analysis.

For more details, see “Creating an Analysis in Variant Analysis” in Help.

The screenshot shows the 'Name your analysis' window, which is part of a four-step wizard. The window title is '4 Analyze'. The main text reads: 'Your analysis is ready to begin. Some analysis take time to run. You will receive an email when the analysis is complete.' There is a 'Summary' section with the following text: '3 case sample(s)', '3 control sample(s)', and '5 custom annotation(s)'. Below this, there is a 'Name your analysis' section with a 'Name' field containing 'My First Analysis' and a 'Description' field containing 'Final disease causing variants'. At the bottom, there are 'Back' and 'Analyze' buttons.

# Explore analysis and review results

Name	Description	From	Created	Samples	Status
Pre-Filtered Analysis		ryp@ingenuity.com	11/10/2015	1/2	active
Inova-003-dominant		soheia@demo.com	10/11/2015	1/2	active
Patient 32 EEC		jnbillaud@ingenuity	09/22/2015	1/1	active
Patient 46 EEC		jnbillaud@ingenuity	09/22/2015	1/1	active
Patient 47 EEC		jnbillaud@ingenuity	09/22/2015	1/1	active
Patient 47 46 32 EEC		jnbillaud@ingenuity	09/22/2015	3/3	active
Adams Oliver Full Geno...		ryp@ingenuity.com	08/25/2015	1/2	active
Family 06-01 only		rupert.yip@qiagen.o	08/11/2015	1/0	active
Family 06		rupert.yip@qiagen.o	08/11/2015	1/3	active
Family 07-01&05		rmad@bioban.o	08/11/2015	2/0	active

Variant Analysis uses the QIAGEN Knowledge Base to link variants in an analyzed dataset to biologically relevant information. Easy to use filters enable rapid identification of variants likely to be causal in the analyzed experiment.

1. Open the analysis under the “My Analyses” tab. The resulting analysis screen displays a series of customizable filters called the “Filter Cascade” and a table of annotated data on the right. Each filter displays 2 numbers. The number to the left indicates the number of variants that remain in the dataset after applying the respective filter and all filters above it. The number to the right indicates the number of distinct genes within which those variants are observed in the analysis.

Chr.	Position	RefSeq	Sample	Variant	Gene Region	Gene Symbol	Protein Variant	Sample	Sample	Translation Impact	SIFT Funct
1	120478125	A	C	SNV	Exonic	NOTCH2	p.F120V	loss: loss	<<<	missense	Damaging
2	170177325	G	C	SNV	Exonic	APF2	p.T16S	loss: loss	<<<	missense	Tolerated
2	179441932	G	A	SNV	Exonic	TTN	p.P13879S, p.P...	loss: loss	<<<	missense	Damaging
3	134251704	G	T	SNV	Exonic	CEP350	p.D135Y	normal: N	<<<	missense	Damaging
4	982720	G	C	SNV	Exonic, Intron	ICU4, SLC25A	p.D498E	normal: N	<<<	missense	Tolerated
4	170386474	A	C	SNV	Exonic	NEK1	p.N648K, p.N67...	normal: N	<<<	missense	Damaging
6	57055318	G	A	SNV	Exonic, non-coding	RAB23	p.P219S	normal: N	<<<	missense	Tolerated
6	170592464	G	A	SNV	Exonic	DLL1	p.R435C	loss: loss	<<<	missense	Damaging
7	2505861	G	A	SNV	Exonic	LPFG	p.E59V	loss: loss	<<<	frameshift	Damaging
9	4171933	C	G	SNV	Exonic	GLIS3	p.E190D, p.E51...	loss: loss	<<<	missense	Damaging
9	36011487	G	A	SNV	Exonic	FANCD2	p.S23F	loss: loss	<<<	missense	Damaging
11	2905353	T	C	SNV	Exonic	CDKN1C	p.K247E, p.K271...	loss: loss	<<<	missense	Damaging
13	25480058	AG		Deletion	Exonic, non-coding	CENPJ	p.S709*	loss: loss	<<<	frameshift	Damaging
16	56765760	T	G	SNV	Exonic, Intron	SLC6A2	p.M1R	loss: loss	<<<	start loss	Damaging
17	74383648	C	T	SNV	Exonic	SPHK1	p.P373L, p.P39C	loss: loss	<<<	missense	Damaging
19	1407367	G	T	SNV	Exonic	APC2	p.R1356L	normal: N	<<<	missense	Damaging
19	71320173	G	C	SNV	Exonic	NR2F1	p.D344E, p.D34...	loss: loss	<<<	missense	Tolerated
19	11210979	G	T	SNV	Exonic	LDLR	p.A45S	loss: loss	<<<	missense	Tolerated
20	44045246	T	A	SNV	Exonic, Intron	PIGT	p.F93	normal: N	<<<	missense	Tolerated
22	25024275	G	A	SNV	Exonic	GGT1	p.V495M	loss: loss	<<<	missense	Tolerated

Variant Analysis Filter Type	Function
Biological Context	Identifies variants with compelling links to disease progression or drug response by specifying relevant biological or clinical terms, including diseases, symptoms, genes, domains, processes, and pathways.
Genetic Analysis	Identifies variants consistent with a particular inheritance pattern, or represented in a certain percentage of case or control individuals in a study.
Common Variants	Keep or exclude variants using allele frequencies in healthy control populations' data.
Predicted Deleterious	Quickly identifies variants in the analyzed dataset with predicted or observed evidence suggesting that they disrupted gene function or expression or are known to be involved in disease.
Cancer Driver	Finds variants within an analyzed dataset with predicted or established association to tumorigenesis or metastasis.
Custom Annotation	Generates filters based on variant, region, or gene annotations imported into Ingenuity Variant Analysis.
Pharmacogenetics	Identifies variants inferred or observed to impact drug response, metabolism, or toxicity based on literature evidence.
Physical Location	Identifies variants on a particular chromosome or within a particular region of a chromosome.
Statistical Association	Excludes or includes variants based on a basic association test that compares allele frequencies between case and control samples.
Confidence Filter	Enables the user to filter out variants of potentially low quality.
User-defined Variants	Enables the user to save and reuse variant sets across analyses.

For more details, see sections corresponding to each filter type in Help.

2. To change the settings of each filter by clicking the “Edit” icon. The following table provides a description of the function performed by each filter type.
3. Remove a filter entirely by clicking “Delete”.

4. Move a filter up or down in the Filter Cascade using the up and down arrows.
5. Add an additional filter by clicking “Add filter” under the filter cascade.

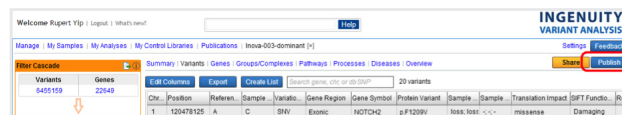
## Sharing an Analysis

Datasets and analyses can be shared with colleagues and collaborators. Recipients that do not already have one, can set up a free, secure account when they receive the shared results.

1. Click the “My Analysis” tab in the top navigation bar
2. Select 1 or more analysis to share and click “Share”
3. In the resulting dialog box, enter the email address(es) of recipients and click “Share”
4. Recipients will receive an automated email informing them there are analysis results awaiting them in their Variant Analysis account. If the recipients don’t have an existing Variant Analysis account, the system will automatically create an account and place the shared analysis therein.

For more details, see “Sharing in Variant Analysis” in Help.

## Publishing in Variant Analysis



1. Click “Publish” in the upper right corner of the analysis to generate an online copy of the analysis to be included as a supplement for any journal article through a stable URL.
2. If the supplement is to be included in an accepted publication, update the supplement title, add the target journal, and click “Release” in the publication status box.

Releasing a dataset gives Ingenuity permission to make the data public and perpetually available via the URL.

**1 Embargo** **2 Embargoed** ✕

Select a unique URL for your free online supplement and embargo it until your article is published. Embargo a copy of this analyzed dataset to enable only you and individuals you specify to access it using a stable URL until your article is published. You may delete or edit it at any time prior to instructing Ingenuity to publicly release it after your article is accepted.

Name \* Yip\_et\_al\_2016

Stable URL [https://variants.ingenuity.com/Yip\\_et\\_al\\_2016](https://variants.ingenuity.com/Yip_et_al_2016)

Title \* Identification of novel mutations in IRS-1 resulting in obesity

Journal Journal of Endocrinology

Description Enter description

Emails Enable these individuals to review analysis:  
Enter email addresses separated by commas

Select from recent emails

Cancel Embargo

The URL can be embargoed so that only specified individuals can access the analysis.

Name	Title	Journal	Published	Status: Embargoed
Yip2016	Identification of novel mutations in IRS-1 resulting in obesity	Journal of Endocrinology	Embargoed	
Enc2015	Expanding the phenotypic spectrum in EP300-related Rubinstein-Taybi syn.	American Journal of Med.	2015	
Arctic	A selective sweep on a deleterious mutation in the CPT1A gene in Arctic pop.	American Journal of Hum.	2014	
PGPten	Rare functionally intriguing homozygous genotypes among Personal Geno...	[public analysis]	2014	
Scott2014Nov2	Characterization of the Genomic Architecture and Mutational Spectrum of a...	Genes	2014	
Scott2014	Characterization of the Genomic Architecture and Mutational Spectrum of a...	Genes	2014	
common-micro	Rare Variant Association with Mucosa among Personal Genome Project (P...	[to be determined, pendin	2013	
Howard2014	Mutation-enriched PGP/Scans4MD	American Journal of Hum.	2013	
Harsanesh2013	Exome sequencing and genome-wide copy number variant mapping revea...	BMC Genomics	2013	
Harsanesh2013	Exome sequencing and genome-wide copy number variant mapping revea...	BMC Genomics	2013	

For more details, see “Publishing in Variant Analysis” in Help.

## Inova Genomes

Inova is a not-for-profit healthcare system based in Northern Virginia that has sequenced over 7000 genomes that are well phenotyped, broadly consented, spanning several disease areas, and ethnically diverse. Variant Analysis users may purchase access to these samples. To learn more, or obtain a quote go to: <https://www.qiagenbioinformatics.com/products/inova-genomes/>.

## Allele Frequency Community

You may participate in the research crowdsourcing initiative, the Allele Frequency Community (AFC) through Ingenuity Variant Analysis. The AFC is a collection of user-contributed, diverse human NGS samples used to generate summarized statistics. Allele frequencies from the anonymized, pool data provides critical context for sequence interpretation in the biomedical community. To learn more about the AFC and the founders go to: [www.allelefrequencycommunity.org](http://www.allelefrequencycommunity.org). To access and opt-in to the allele frequency community simply click the "opt-in" option upon first login or click the "Settings" link in the upper right of the Ingenuity Variant Analysis application to opt-in to the AFC.

## FAQ

Variant Analysis Filter Type	Function
Are data secure in Variant Analysis?	Ingenuity Systems has passed a Health Insurance Portability and Accountability Act (HIPAA) audit, confirming that Ingenuity's data center and Ingenuity Variant Analysis are in compliance with relevant Federal Regulations of the US. Thus, Ingenuity securely serves clients such as clinical researchers, molecular pathologists and medical geneticists with strict controls for patient security. For more details, see the Ingenuity privacy policy at <a href="http://www.ingenuity.com/privacy-policy">www.ingenuity.com/privacy-policy</a> .
What file formats are supported for upload to Variant Analysis?	Variant Analysis currently supports Variant Call Format (VCF), Genome Variation Format (GVF), and Complete Genomics' files (Var, VarMaster, High confidence junction, etc.). For more details, see "Variant Analysis file format" in Help.
Can multiple samples be analyzed together?	Yes. Any number of cases and optional controls can be analyzed together
How can large datasets (i.e., more than 10 samples) be uploaded?	Large datasets can be securely uploaded via the High-volume uploader link, available within the upload window.
What species are accepted for analysis in Variant Analysis?	Variant Analysis accepts variants aligned and called relative to a human reference genome (Hg18, Hg19, and Hg20) . For more details, see "Creating an Analysis in Variant Analysis" in Help.
How many samples can be included in a single analysis?	There is no limit to the number of cases or controls that can be analyzed. To accommodate large studies, the graphical icons that indicate function, genotype, copy number, call quality, etc., will shrink to colored vertical lines that indicate loss, normal, or gain function. This way, quick comparison of frequency and impact of variants between case and control samples is still possible.
Can RNA-Seq data be integrated with DNA re-sequencing data?	Yes. Use the Custom Annotation Filter to create filters based on RNA-Seq data imported into Variant Analysis. For more details, see "Custom Annotation" in Help.
Can variant sets be saved for reuse across analyses?	Yes. Click "Create list" above the variant table, give the list a name, and click "Save". For more details, see "User Defined Variants" in Help.
Is it possible to define terminology for describing variants?	Terminology in Variant Analysis is aligned to current standards, including HGVS. For more details, see "Glossary for Variant Analysis" in Help.
Can analyses from Variant Analysis be shared?	Yes. Variant Analysis is licensed on a per-sample basis. One advantage of this cost structure is that sharing samples and analysis results with colleagues and collaborators in the same group is free of charge. For more details, see "Sharing in Variant Analysis" in Help.
Can the Ingenuity Variant Analysis software be cited in a scientific article?	Yes. For more details, see "Citing Ingenuity Variant Analysis" in Help.



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For further support with technical difficulties please contact

Ingenuity Customer Support:

**[support-ingenuity@qiagen.com](mailto:support-ingenuity@qiagen.com)**

+1 (650) 381-5111

[www.ingenuity.com](http://www.ingenuity.com)

Ingenuity Variant Analysis is intended for molecular biology applications. This product is not intended for the diagnosis, prevention, or treatment of a disease.

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