Ingenuity® Variant Analysis™ for Ion Reporter™ Software Quick-Start Guide

For accessing and using Ingenuity Variant Analysis through Ion Reporter Software

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Ingenuity® Variant Analysis™ is a web-based application that combines analytical tools and integrated biological and chemical content to rapidly identify and prioritize compelling causal variants based on published biological evidence and user knowledge of disease biology. Ion Reporter Software enables sequence reads from the Ion PGM™ and Ion Proton™ Systems to be automatically uploaded from Torrent Suite™ Software for analysis. This Quick-Start Guide summarizes how to access the Ingenuity Variant Analysis plug-in for Ion Reporter, transfer data files, and perform and share analyses.

Before using Variant Analysis, participate in the free online live events covering the basic and intermediate functionalities. For more information, view the upcoming schedule, and register for upcoming events, visit the "Training" tab at www.ingenuity.com/products/variant-analysis. Pre-recorded and written materials are available at http://ingenuity.force.com/variants/VariantTutorials. Additionally, recorded scientific seminars designed to answer specific research questions can be viewed under the "Resources" tab of the above mentioned product page. Upcoming scientific seminars are listed at www.ingenuity.com.

The "Help" function in Variant Analysis is a comprehensive, keyword-searchable resource, designed to quickly find necessary information and instructions. Click "HELP" at the top of the Variant Analysis application or visit http://ingenuity.force.com/variants/VariantTutorials.

Accessing Variant Analysis

Anyone with an active Ion Reporter account can access Variant Analysis through the integrated plug-in. No additional login or registration is required.

An analysis of 1–3 samples is performed in Ion Reporter to identify variants. After viewing and editing the analysis results, the Variant Analysis plug-in can be initiated by selecting it below the variant table.

Variant Analysis can be accessed directly at https://variants.ingenuity.com/va/ using your Ion Reporter username. Request your password to be reset at https://apps.ingenuity.com/isa/account/forgotpassword or contact Customer Support at support@ingenuity.com or +1 (650) 381-5111.

Uploading data files to Variant Analysis

Variant Analysis is a web-based application delivered via the Ingenuity secure private cloud environment. Selecting the Variant Analysis plug-in initiates the secure transfer (with 256 bit AES encryption both in transit and at rest) of called variant files to Variant Analysis. The transfer is completed in a few minutes. A transfer screen tracks the transfer and processing of data. Upon completion, the analysis in Variant Analysis will appear embedded within the Ion Reporter window. In addition, Variant Analysis sends an email notifying the availability of the analysis.

Previewing an analysis

New and existing users of Variant Analysis can preview results of an analysis free of charge, prior to activating the samples of the analysis.

The preview includes access to the full functionality of Variant Analysis, excluding the publication and export features, for 30 min. The preview can be shared with collaborators or colleagues via email. After 30 min, a payment is required to further access the analysis. Variant Analysis enables payment for immediate analysis activation through the Life Technologies e-commerce system or by entering an Ingenuity activation code.

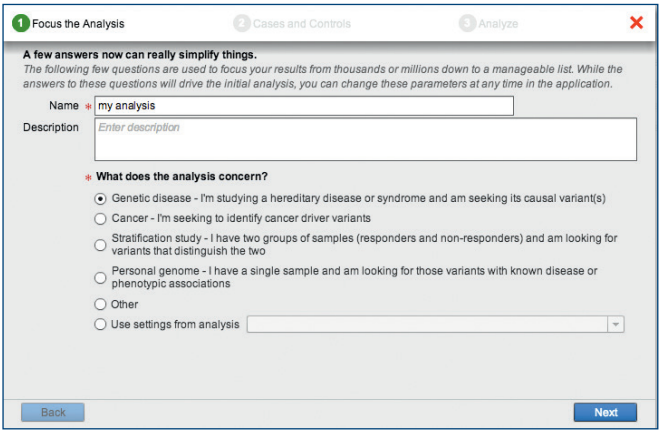
For more details, see “Activating samples in Variant Analysis” in Help.

Creating an analysis

The Variant Analysis plug-in in Ion Reporter automatically generates an analysis. The following are instructions to re-analyze samples in Variant Analysis. Specify 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.

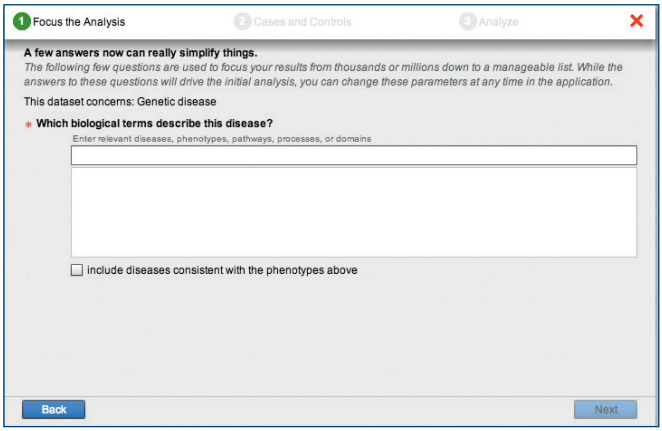
1. **Click the "My Analyses" tab in the top navigation bar, and then click "Create".**

2. **Provide a name for the analysis file and select a study type.**

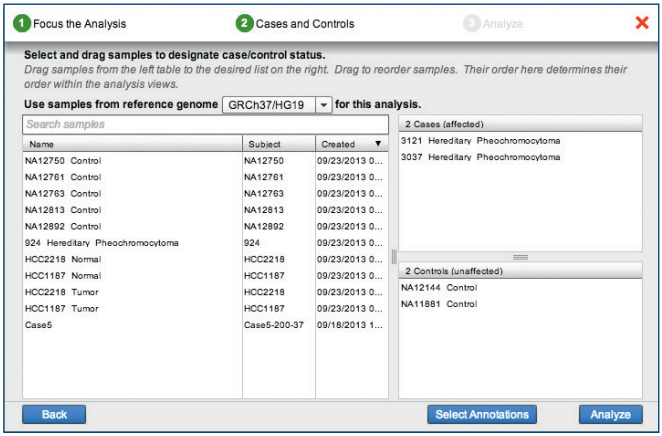


3. **Enter terms that describe the phenotype of interest.**

   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.



4. **Select samples for each of two distinct groups: one or more cases and optional controls.**



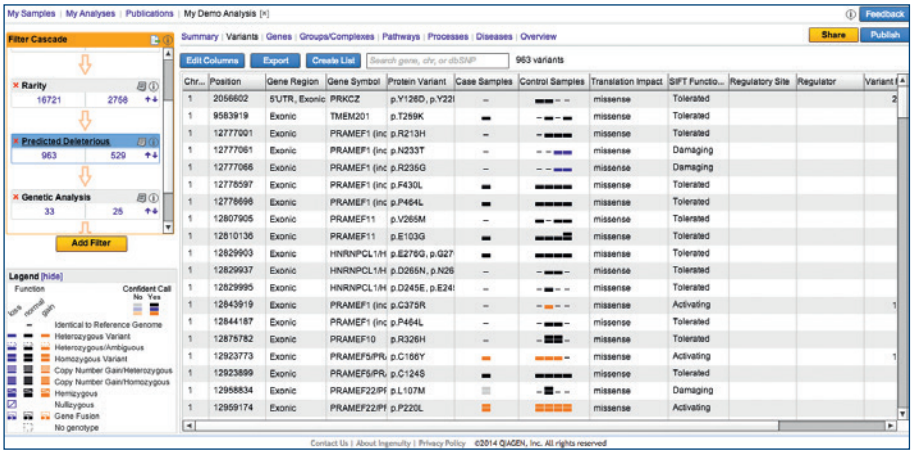
5. **Click "Select annotations" or "Analyze" to initiate the analysis process.**

   "Select annotations" allows you to first select the annotations associated with the samples chosen for analysis. Variant Analysis requires a few minutes to create an analysis of small studies. Large studies with hundreds or thousands of samples can take longer. Closing the window or application once the screen "Performing Variant Analysis" appears will not stop the process. An email with a link to the new analysis is sent to the user when the process is done.

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

Linking variants to biology

Variant Analysis uses the Ingenuity 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 table of annotated data and a series of customizable filters called the "Filter Cascade" on the left. 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.

2. **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.**

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 heritance pattern, or represented in a certain percentage of case or control individuals in a study.
Common Variants	Excludes variants observed in healthy control individuals more frequently than the disease or phenotype prevalence of interest.
Predicted Deleterious	Quickly identifies variants in the analyzed dataset with predicted or observed evidence suggesting that they disrupted gene function or expression.
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.

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.**

Exporting activated analysis results to Ion Reporter

After identifying the most promising variants in Variant Analysis, filter selections and Ingenuity annotations can be exported back to Ion Reporter. The analysis must be activated to export analysis results to Ion Reporter.

1. **Click "Export" and select the "Ion Reporter" option.**

   After successful export, a new column labeled "Ingenuity" will appear in the main Summary section of the results table in Ion Reporter.

   This column contains the assessment by Variant Analysis of each variant based on published literature.

2. **"Ingenuity Variant Analysis" can subsequently be used as an option in setting up filter chains in Ion Reporter.**

Sharing and publishing 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.

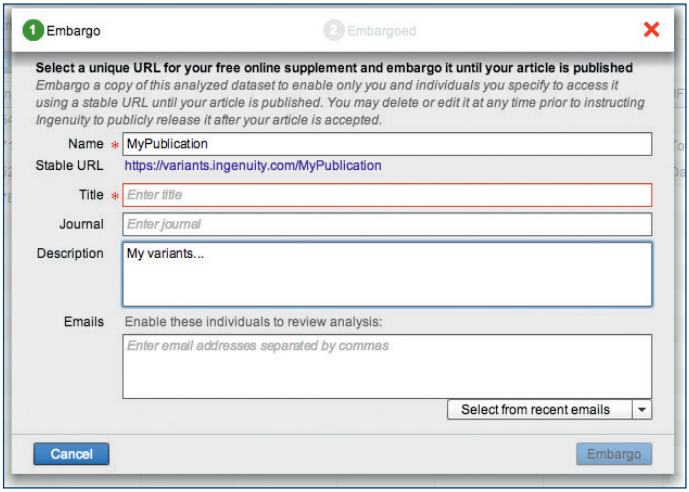
1. **Click the "My Analyses" tab in the top navigation bar.**
2. **Select 1 or more analyses to share and click "Share".**
3. **In the resulting dialog box, enter the email addresses of recipients and click "Share".**

   Recipients will receive an automated email informing them to access the shared analysis in Variant Analysis.

   For more details, see "Sharing in Variant Analysis" in Help.

4. **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.**

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



5. **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 implies permission to make the data public and perpetually available via the URL.

   For more details, see "Publishing in Variant Analysis" in Help.

FAQ

Question	Answer
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 www.ingenuity.com/privacy-policy.
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. 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 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.

For further support with technical difficulties please contact Ingenuity Customer Support:
support@ingenuity.com
+1 (650) 381-5111
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.

For up-to-date licensing information and product-specific disclaimers, see the respective Ingenuity product site. Further information can be requested from support@ingenuity.com or by contacting your local account manager.