VariantGrid

CCB ACRF Cancer Genomics Facility

Oct 10, 2019

OVERVIEW:

VariantGrid is an open source variant database and web application for analyzing genetic data.

CHAPTER

ONE

INTRO

VariantGrid has a number of installations. Please visit the individual sites for login/registration details.

1.1 Cloud servers

- variantgrid.com - Research cloud server
- runx1db - Rare disease exome sharing
- Shariant - Australian Genomics variant classification sharing platform

1.2 Private server

There is a VariantGrid private server inside SA Pathology, the public pathology provider to the South Australian Health.

The advantages of a private server are being restricted to a private intranet, and being able to analyse private patient data without worrying about it being on the cloud.

To install a local copy of VariantGrid, please see the GitHub page.

CHAPTER

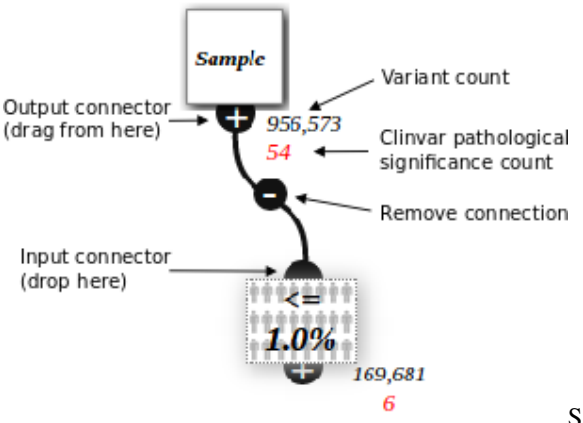
TWO

ANALYSIS INTRO

Create custom variant filters by connecting together nodes representing sources or filters of variants. See analysis nodes

Other variant databases allow similar creation of filters, but VariantGrid can constuct nodes in real-time, enabling rapid exploration of large and difficult genomic data sets.

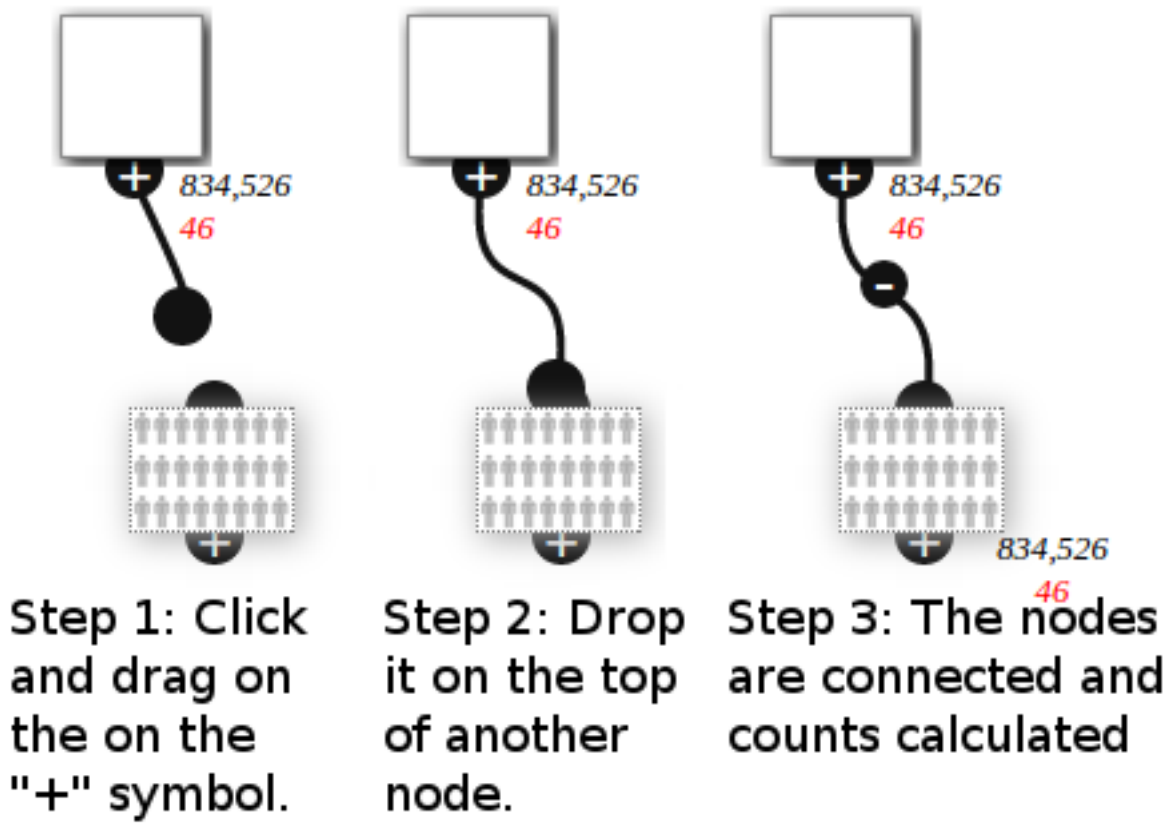
2.1 Analysis Nodes



Sample Node connected to a Population Filter Node

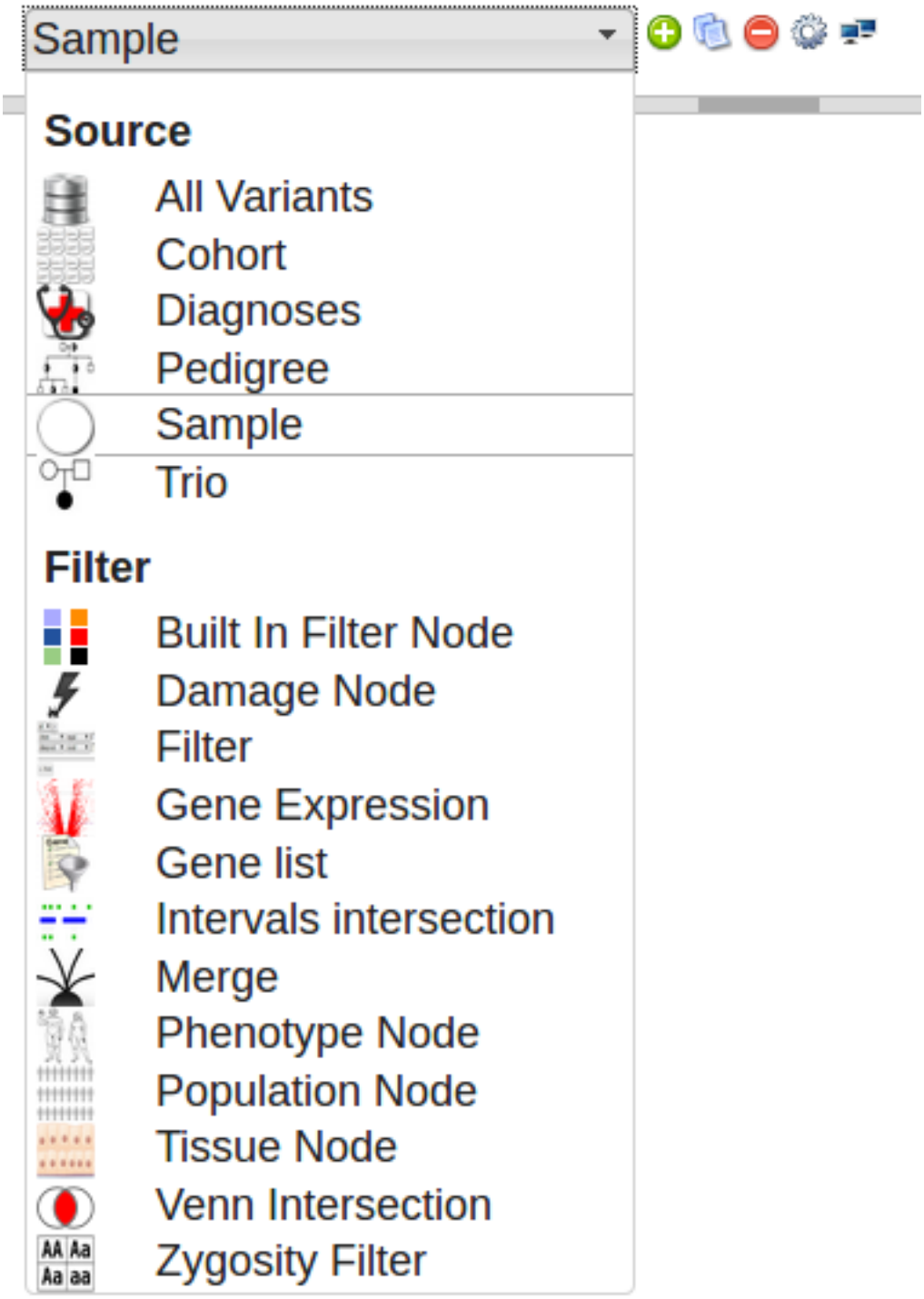
The top node is configured to show a particular patient exome (from an uploaded VCF).

These variants are then filtered to those that are less than 1% of the population.



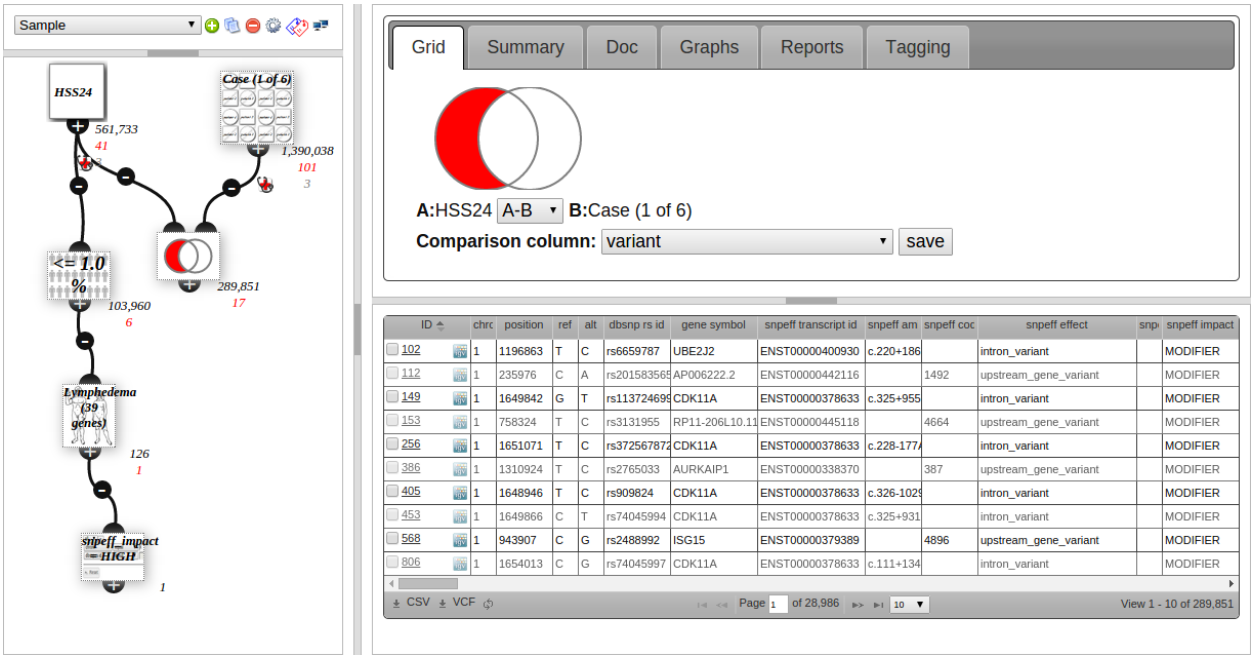
Connecting Nodes

To add a node, select the node type from the drop down menu in the top left of the screen and click the add button



Click and drag a node to move it around. You can select multiple nodes by drag-selecting a box around them. This allows you to copy, delete or move them as a group. Delete selected nodes by pressing DELETE, or click the delete button.

2.2 Analysis screen



The screenshot above shows the VariantGrid analysis screen. The node graph is on the left part of the screen, showing the user built filters.

Click a node to select it. This loads the node editor (top right) and a grid of the variants (see section below) in the node (bottom right).

Clicking on the node loads this editor window. The node editor is different depending on the type of node.

2.3 Analysis Grid

The 1st column (ID) is special and contains a check box, a numbered link and an IGV logo. The check box is used to select rows manually. The link loads detailed information about that variant above the grid. The IGV link will view the locus in IGV (loading bam files associated with samples). See IGV Integration page. Clicking on a row highlights it. Select the "tagging" tab, then click on a label to tag/colour the row.

CHAPTER

THREE

ANALYSIS NODES

3.1 Source Nodes

Provide a source of variants

3.1.1 All Variants

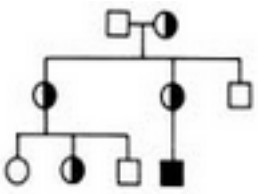
All variants in the database.

3.1.2 Cohort

A collection of related samples, eg “control group” or “poor responders”

3.1.3 Classifications

3.1.4 Pedigree



Variants from family samples filtered by genotype according to inheritance models

3.1.5 Sample

A sample, usually one genotype (patient, cell or organism) with a set of variants.

3.1.6 Trio

Mother/Father/Proband - filter for recessive/dominant/denovo inheritance

3.2 Filter Nodes

These nodes filter variants connected to the top of them

3.2.1 Built In Filter

Built in filters used in node counts eg High or Moderate Impact / OMIM / ClinVar Pathological

3.2.2 Damage

Filter to damage predictions

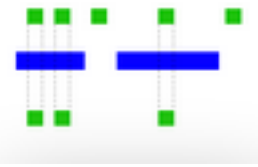
3.2.3 Filter

Filter based on column values

3.2.4 Gene List

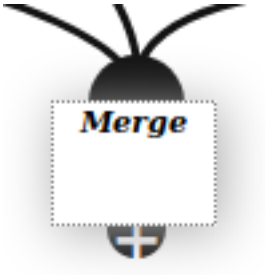
Filter to a list of gene symbols

3.2.5 Intervals Intersection



Filter based on intersection with genomic ranges (eg .bed files)

3.2.6 Merge



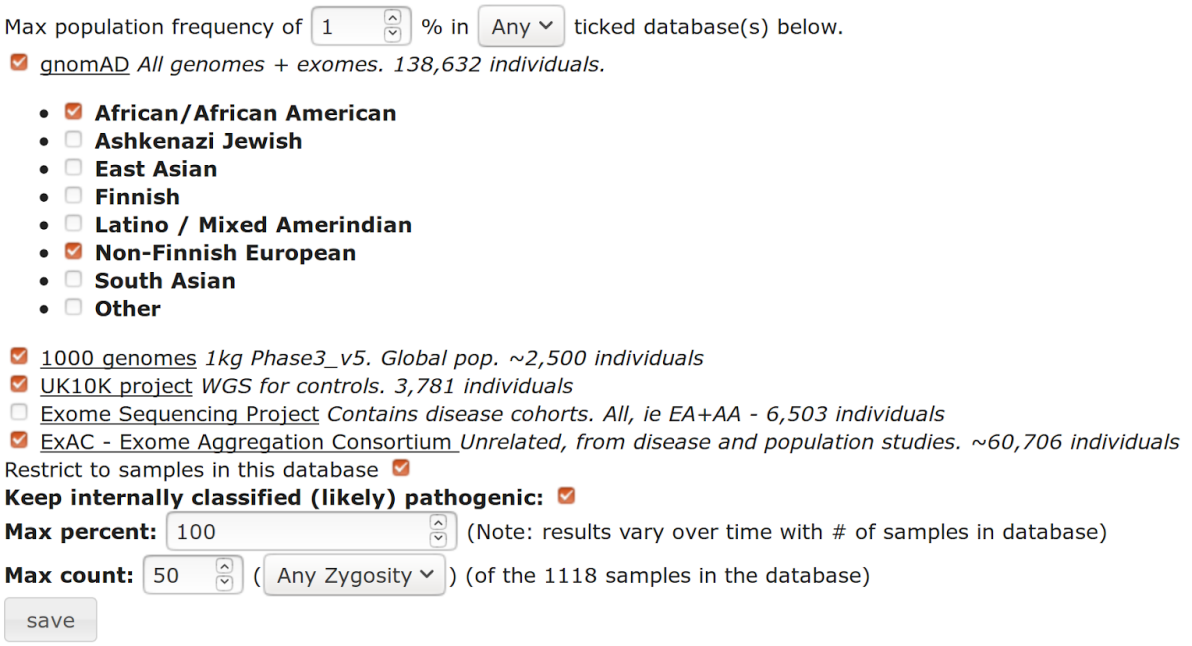
Merge variants from multiple sources

3.2.7 Phenotype

Filter to gene lists based on ontology keywords

3.2.8 Population

Filter on population frequencies in public databases (gnomAD/Exac/1KG/UK10K) or number of samples in this database.



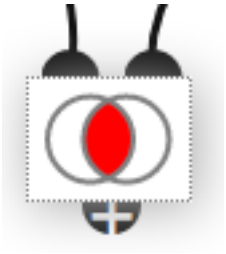
3.2.9 Tags

Filter variants to those that have been tagged

3.2.10 Tissue Expression

Filter based on tissue specific expression (from Human Protein Atlas)

3.2.11 Venn



A filter based on set intersections between parent nodes

3.2.12 Zygosity

Compound HET and other Zygosity filters

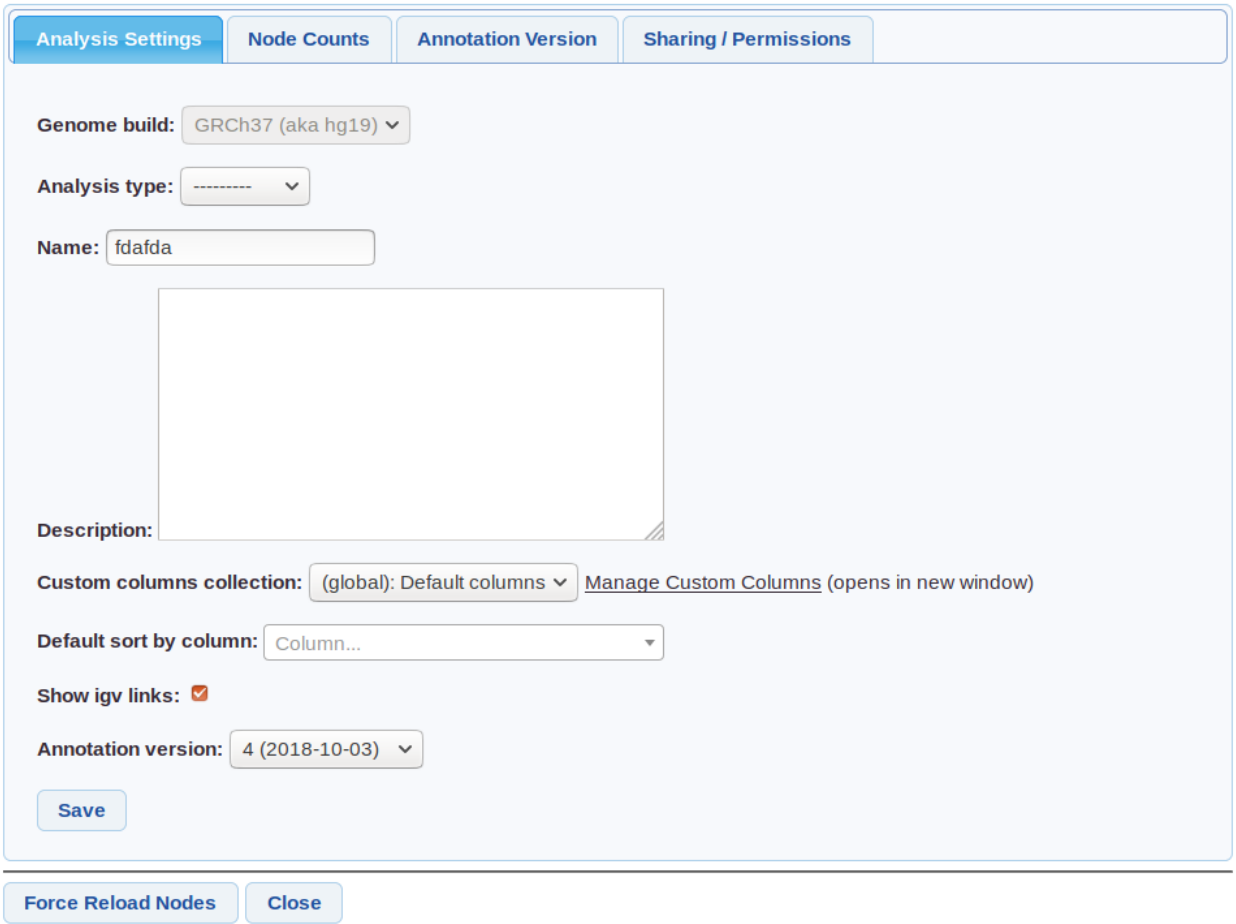
CHAPTER

FOUR

ANALYSIS - ADVANCED

4.1 Analysis settings

In an analysis click the  Settings icon to open the analysis settings page.



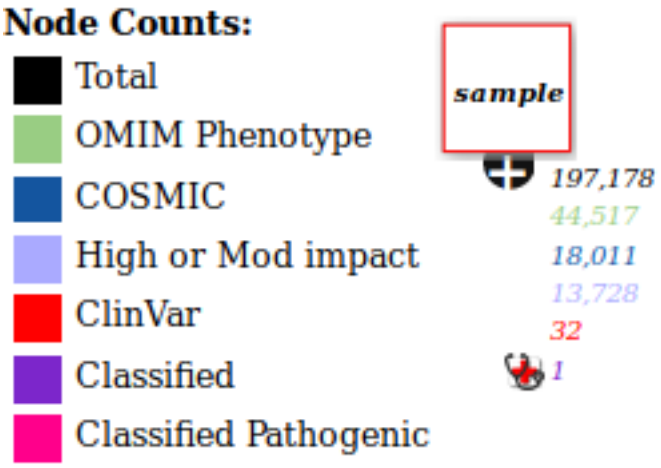
Analysis settings screenshot

- **Genome build** - Cannot be changed. Only data (eg VCF samples) from this build can be used in the analysis.
- **Analysis type** - One of (Singleton/Cohort/Trio/Pedigree) set at creation if using an auto-analysis.
- **Custom columns** - Columns to use - from *customise columns*. Default set in *user settings*

- **Default sort by column** - Can be used for example to make the grid always sort by gene.
- **Annotation Version** - The *Annotation Version* used.

4.2 Node Counts

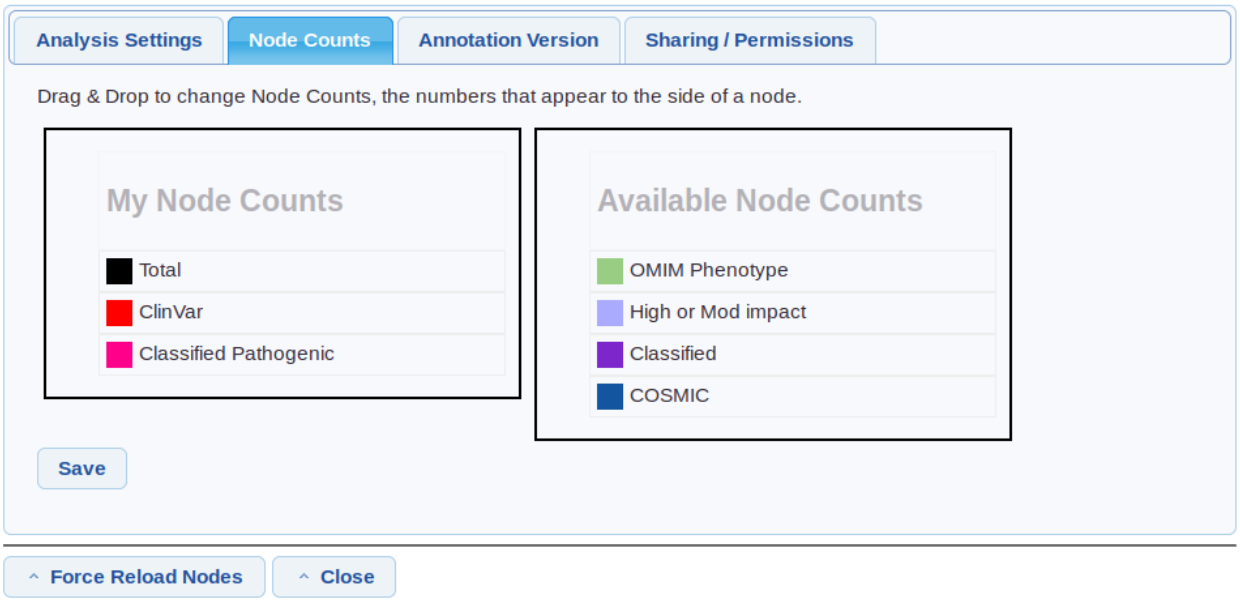
The numbers below a node are counts of variants that meet a certain criteria. The colours correspond to names in bottom left hand legend, eg in the image below, there are 32 ClinVar (Likely) Pathogenic variants in that node.



Node with counts

Click on a count to load the variants in the node that meet that criteria, eg clicking on the red 32 would just load the ClinVar variants.

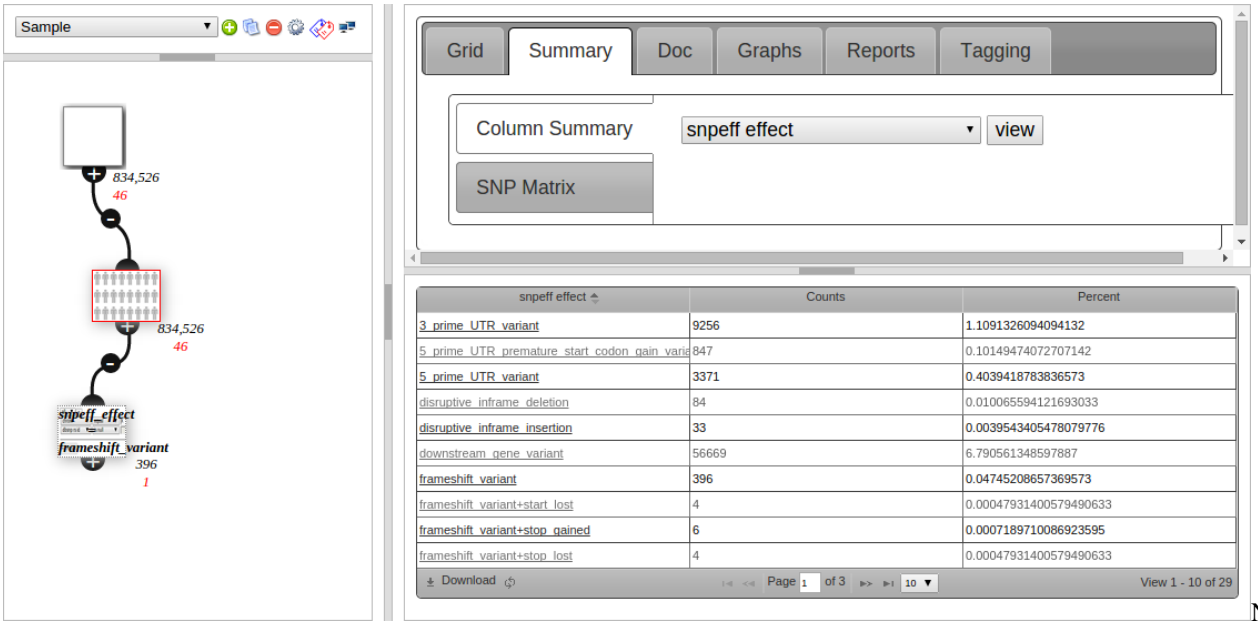
To edit which node counts are shown, open analysis settings, then select the "node counts" tab.



Settings/Node counts

Drag and drop the node counts to show/hide them and change the order.

4.3 Column Summary



Node Summary

The second tab (Summary) is used to view what values are in a column. Qualitative data is counted and shown in a grid, such as snpEFF Effect in the screenshot below:

Clicking on the link in the 1st column creates a child node filtering to that value. This is useful for getting an overview then drilling down into your data.

The screenshot shows 396 entries under “frameshift variant”, and the filter node created underneath the current (red bordered) node, which is configured to filter to snpeff_effect = frameshift variant, and also has 396 variants after filtering.

Quantative data (numbers, such as for the af_1kg column (1000 Genomes Alt Frequency)) is shown as a box-plot.

CHAPTER

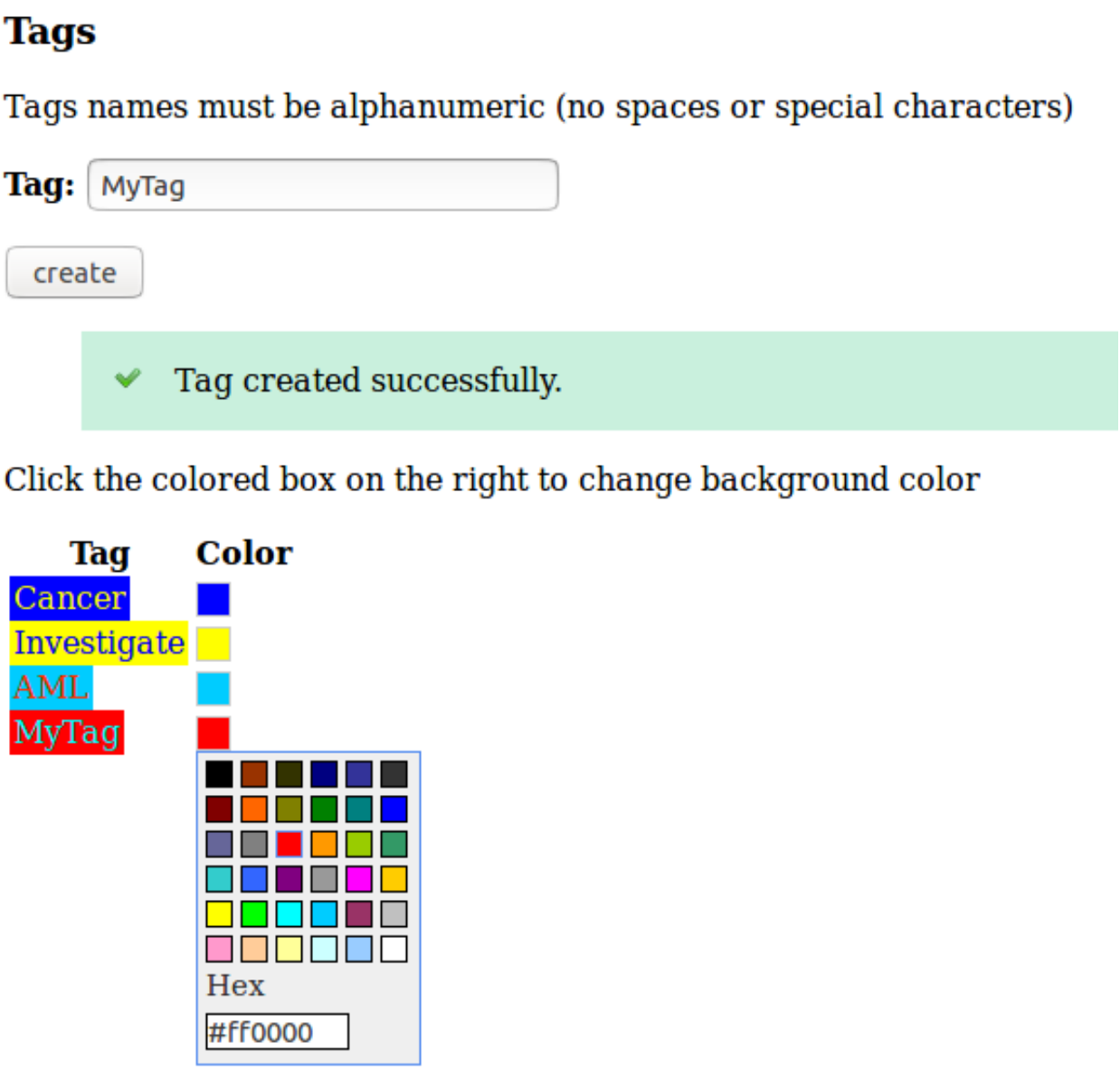
FIVE

VARIANT TAGGING

A tag is a label (such as “Cancer” or “Investigate”) which you can use to label and track variants in an analysis.

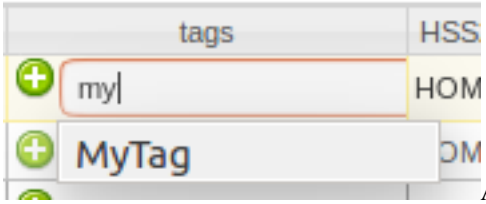
5.1 Create tags

Menu: **[settings] -> [tags]**



5.2 Tagging variants

In an analysis, click the Add icon in the "tags" column then auto-complete your tag.



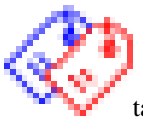
Adding a tag

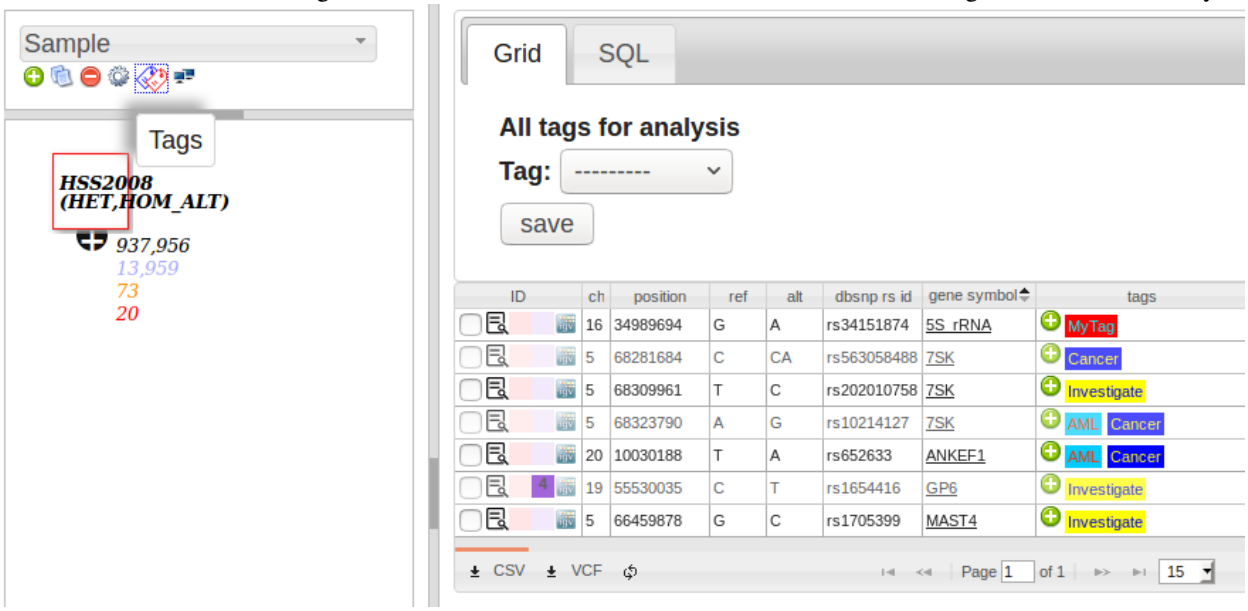
To remove a tag - clicking on the tag. The tag will grow in size, and a delete symbol will appear. Click it to remove

the variant tag.

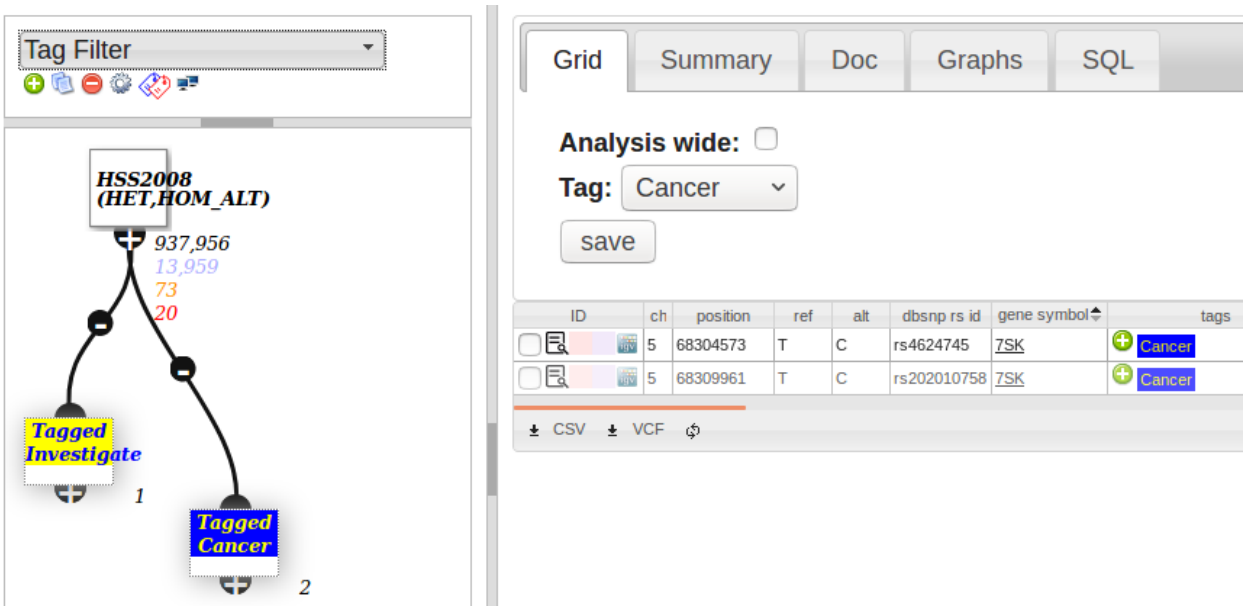
Removing a tag

5.3 Using tags

Click the  tag icon on the toolbar to view all Tags in an analysis



To filter to specific tags - add a tag node, and use it like any other node to filter variants to just those that have been tagged.



You can view all tagged variants on a page, via menu: **[analysis] -> [Tagged Variants]]**

CHAPTER

SIX

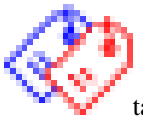
ANALYSIS CLASSIFICATION

Recommended workflow to create a *classification* from a variant in an analysis:

1. *Tag* the variant with the "RequiresClassification" tag.

ID	ch	position	ref	alt	dbsnp rs id	gene symbol	tags	HSS2008	AD	AF	DP	GQ	PL	HSS2009	AD	AF	D
2	12	49433599	T	G	rs147706410	KMT2D		HET	31	47.6	null	null	0	HET	24	44.4	n
2	12	49428694	T	C	rs146044282	KMT2D		HET	56	43.7	null	null	0	HET	56	49.1	n
	6	10410466	T	G	rs776792762	TFAP2A	RequiresClassification	HET	3	33.3	null	null	0	.	2	12.5	n

CSV VCF Page 1 of 1 15

1. Click the  tags button, then then "Classification" tab.
2. Select the sample, then click the **[classify]** button.

CHAPTER

SEVEN

KARYOMAPPING

7.1 Background

We handle the simpler case of a *Trio* with an affected child (ie proband/mother/father).

Variants are assigned to the following bins

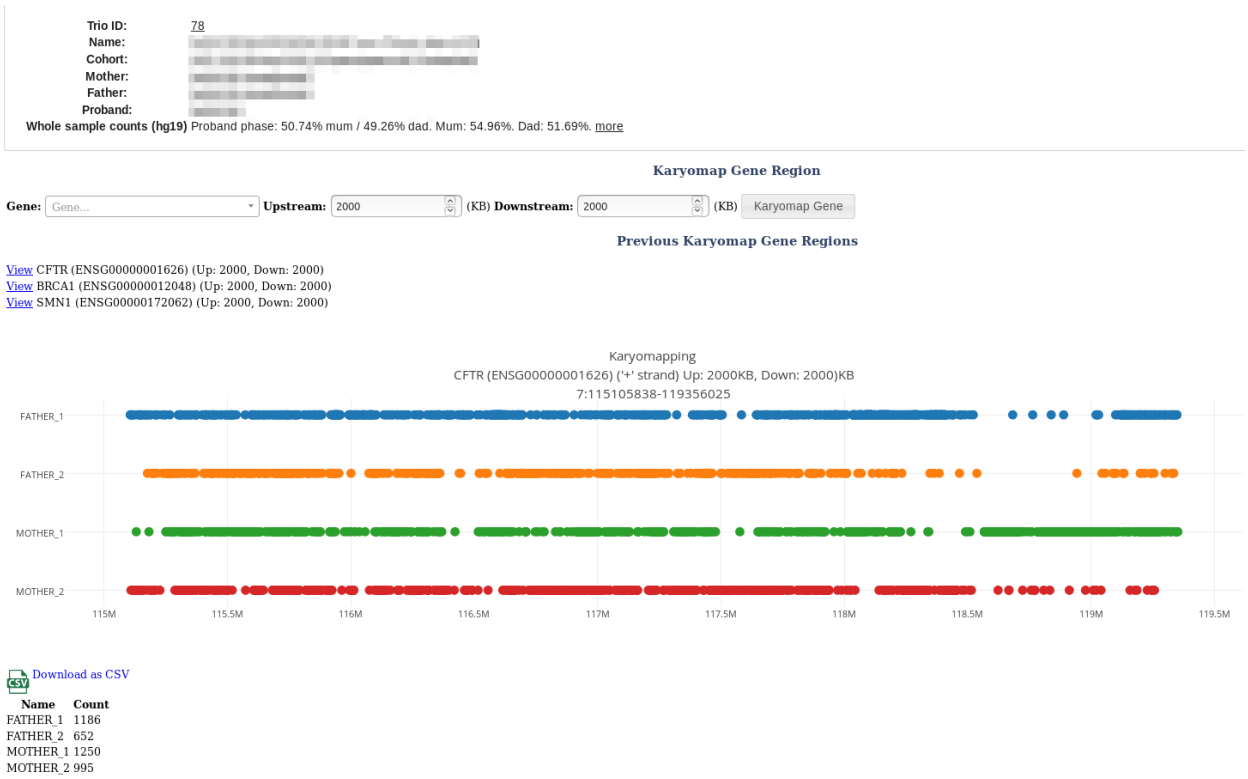
F1ALT: Paternally inherited, in phase with affected child, ALT variant. F1REF: Paternally inherited, in phase with affected child, REF variant. F2ALT: Paternally inherited, out of phase with affected child, ALT variant. F2REF: Paternally inherited, out of phase with affected child, REF variant.

And vice versa for the mother. The only variants that fall into each of these situations are:

7.2 Gene analysis

Menu: **[analysis]** -> **[karyomapping]**

Enter a gene name and click **[Karyomap Gene]** button.



7.3 Genome-wide analysis

A genome wide karyomap count is performed when you create a trio. This is useful for finding sample mixups.

This is summarised as *Proband phase: 50.74% mum / 49.26% dad. Mum: 54.96%. Dad: 51.69%.* and is visible on the gene analysis screenshot above and the *Trio* page.

Proband phase shows the child's marker percentage from each parent. Mum%/Dad% = Percent of parent markers that are in phase in proband.

Here are some examples for various Trios:

As a rough rule, you'd expect a minimum of 40% for an actual child.

CHAPTER

EIGHT

ANNOTATION DETAILS

Annotation refers to all of the information about a variant, it is made from different components, including:

Variant-level annotation: Information specific to a base change. Examples include computational predictions and effects, and existing database entries (such as population frequency for the variant)

Gene-level annotation: Information about the gene (from RefSeq/Ensembl + other sources), matched from the variant's assigned transcript_id.

ClinVar: Clinical variant classifications from ClinVar

To see a description of each field, use menu: **[annotation] -> [descriptions]**

Annotation is shown on the variant details page, and in an *analysis*, where it is used in filters and shown on the grid (see *customise columns*)

8.1 Variant Level Annotation

The first time we see a variant, it is annotated by the variant annotation pipeline.

8.2 Annotation Versions

Each annotation component above is versioned and can be upgraded separately by the site administrator. To see the versions via menu: **[annotation] -> [versions]**

VariantGrid can store multiple annotation versions, which allows us to load historical analyses which return the same results as when they were first analysed, as well as updating from new sources regularly.

8.3 IVAT

VariantGrid uses IVAT developed by Jinghua (Frank) Feng from the CCB ACRF Cancer genomics facility.

8.3.1 SACGF Tiers

Tier 1

Novel variants, with evidence of being strongly damaging, and without any evidence of being artificial:

- Not in dbSNP, 1KG, UK10K, ExAC or ESP

- HIGH or MODERATE snpEFF impact, or mutating at branch point, at miRNA binding site, or at transcription factor binding site
- For a SNV: GERP > 4 or CADD > 30
- For an INDEL: not in LowComplexRegion
- Not in SegmentDup region
- No multi-ALT alleles were called

Tier 2

Extremely rare variants, with evidence of being strongly damaging, and without any evidence of being artificial:

- Not Tier 1
- Minor allele frequency (MAF) < 0.05% in 1KG, UK10K and ExAC.
- HIGH or MODERATE snpEFF impact, or mutating at branch point, at miRNA binding site, or at transcription factor binding site
- For a SNV: GERP > 3 or CADD > 20
- For an INDEL: not in LowComplexRegion
- Not in SegmentDup region
- No multi-ALT alleles were called

Tier 3

Very rare variants, with evidence of being potentially functional, and without any evidence of being artificial:

- Not Tier 1 or 2
- MAF < 0.2% in 1KG, UK10K and ExAC.
- HIGH, MODERATE or LOW snpEFF impact, or mutating at branch point, at miRNA binding site, or at transcription factor binding site
- For a SNV: GERP > 2 or CADD > 20
- For an INDEL: not in LowComplexRegion
- Not in SegmentDup region
- No multi-ALT alleles were called

Tier 4

Rare variants, with evidence of being potentially damaging. They can locate within the SegmentDup regions, and hence are with increased chance of being artificial:

- Not Tier 1, 2 or 3
- MAF < 0.5% in 1KG, UK10K and ExAC.
- HIGH, MODERATE or LOW snpEFF impact, or mutating at branch point, at miRNA binding site, or at transcription factor binding site
- For a SNV: GERP > 2 or CADD > 20

- For an INDEL: not in LowComplexRegion

Tier 5

Uncommon variants with potential damage effect, and can located in SegmentDup and LowComplexRegion and hence with significantly increased chance of being artificial:

- Not Tier 1, 2, 3 or 4
- MAF < 1% in 1KG, UK10K and ExAC
- Satisfying ***any one** of the three criteria below:
  - Annotated with HIGH, MODERATE or LOW snpEFF impact (aka. altering the exon or splice region)
  - Altering splicing branchpoint, miRNA binding site, or transcription factor binding site
  - GERP > 2 or CADD > 20

Tier 6

- Not Tier 1, 2, 3, 4 or 5

Notes:

A variant is classified as Tier 6, when all your samples are HOM-ALT at the variant and that ALT allele is common in 1KG, UK10K and ExAC (i.e. The frequency of the ALT allele is > 0.5 in anyone of 1KG, UK10K and ExAC). This applies before all the tiering above. From a trio sequenced with the Medical Exome Capture on our NextSeq machine in September 2016, below are the numbers of variants (called by GATK, mostly germline) for each tier:

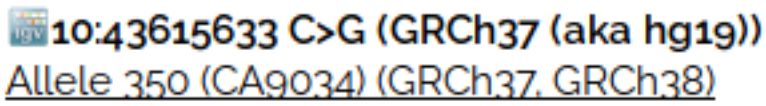
CHAPTER

NINE

VARIANT DETAILS

This page shows the annotation and other information about a variant.

The top of the page has an IGV link, and a link to the allele for this variant:



An allele is genome build independent - ie hg19 and hg38 variants for same change point to same allele. The ID (CA9034) is from the ClinGen Allele Registry

9.1 Classifications

Variant Details - Classification section

This shows internal *classifications* for an allele (may have been classified against a different genome build)

The far right column contains *Classification Flags*

9.2 Transcripts

Variant annotation is calculated for each transcripts overlapping a variant. You can select each of the different transcripts to change which is being displayed.

9.3 Samples

At the bottom of the page is a grid of samples that contain the variant (and the zygosity and read information). Only samples you have permissions to view are shown, but a warning will be shown informing you that samples you don't have permission to see exist.

CHAPTER

TEN

REPRESENTATIVE TRANSCRIPT

SnpEff calculates the damage effects for each transcript. The representative transcript is chosen as:

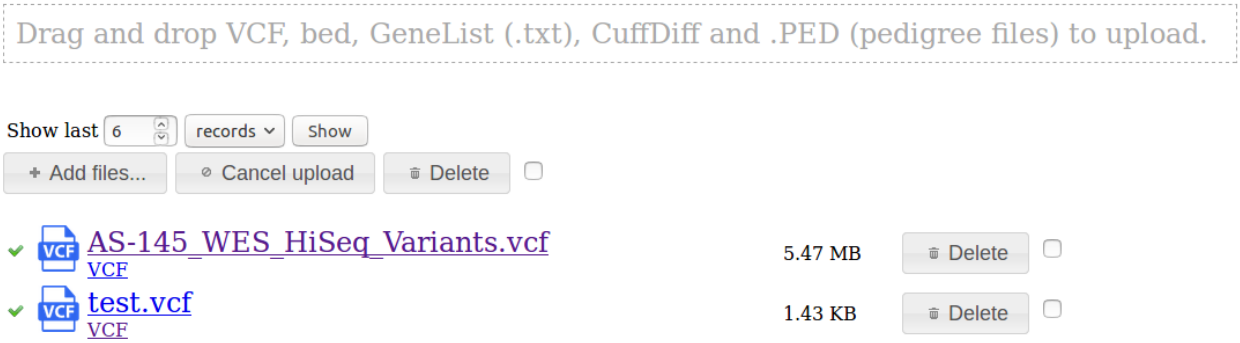
1. The most damaging transcript
2. If equally damaging, the canonical transcript defined by Ensembl is selected
3. If no canonical transcript exists, the longest transcript is selected. If more than one canonical transcript exists, the longest canonical transcript is selected.

CHAPTER

ELEVEN

UPLOADING DATA

Menu: **[data] -> [upload]**



You can either drag & drop files onto the page, or by selecting the **[Add Files]** button.

After the file has been transferred to the server, a spinning icon () will appear as the file is processed. The large link (eg "AS-145_WES_HiSeq_Variants.vcf") takes you to the import processing page if you'd like to monitor the progress.

Once it has been successfully imported, a link will appear beneath the file (eg the "VCF" links above) allowing you to jump to the data page for this file.

CHAPTER

TWELVE

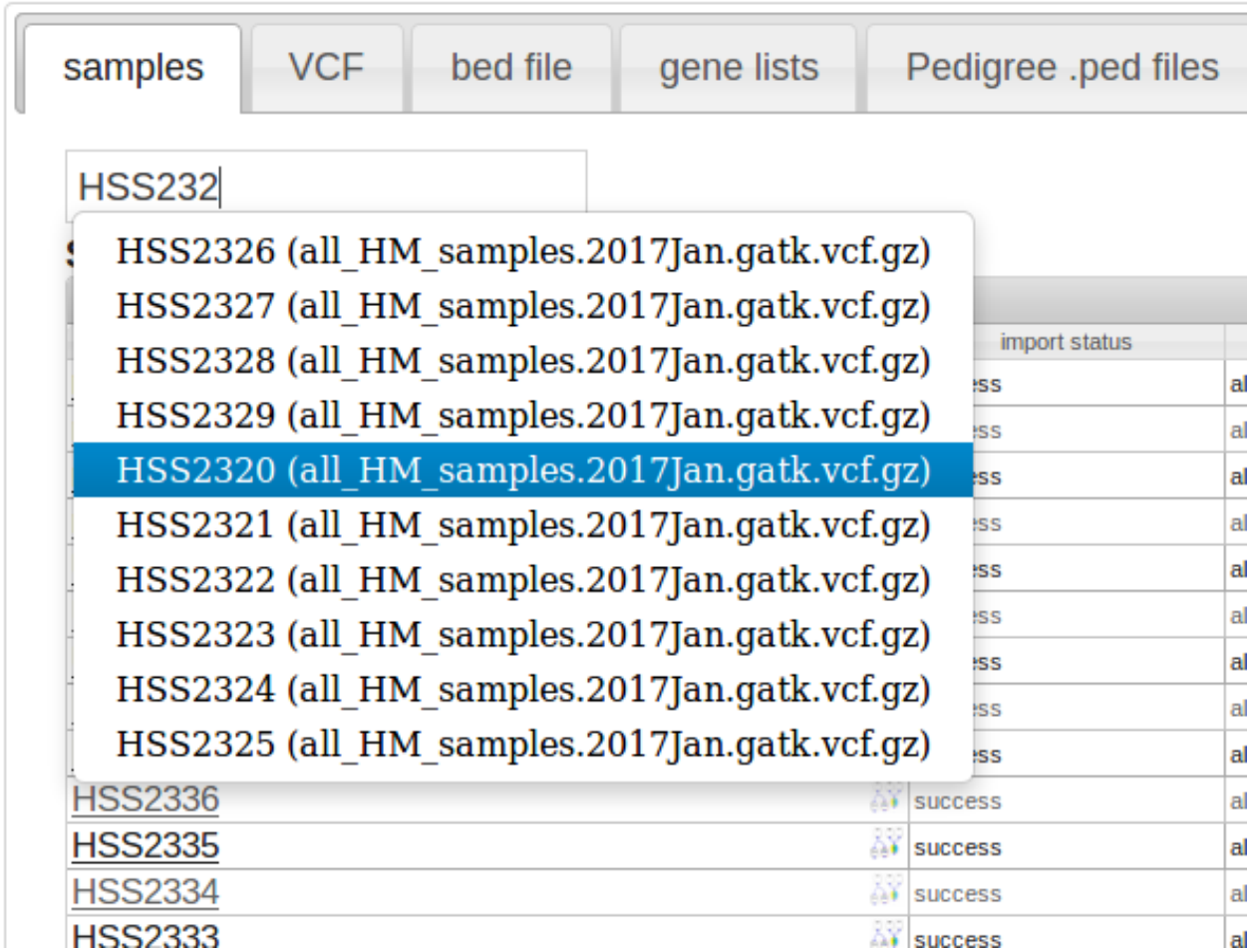
MANAGING DATA

Menu: **[data]**

The data page displays all of your uploaded data such as (VCFs, Bed files, Pedigree Files etc)

Data is displayed in grids, with each data type in a separate tab.

You can enter parts of the name into an autocomplete search box to quickly find your files:



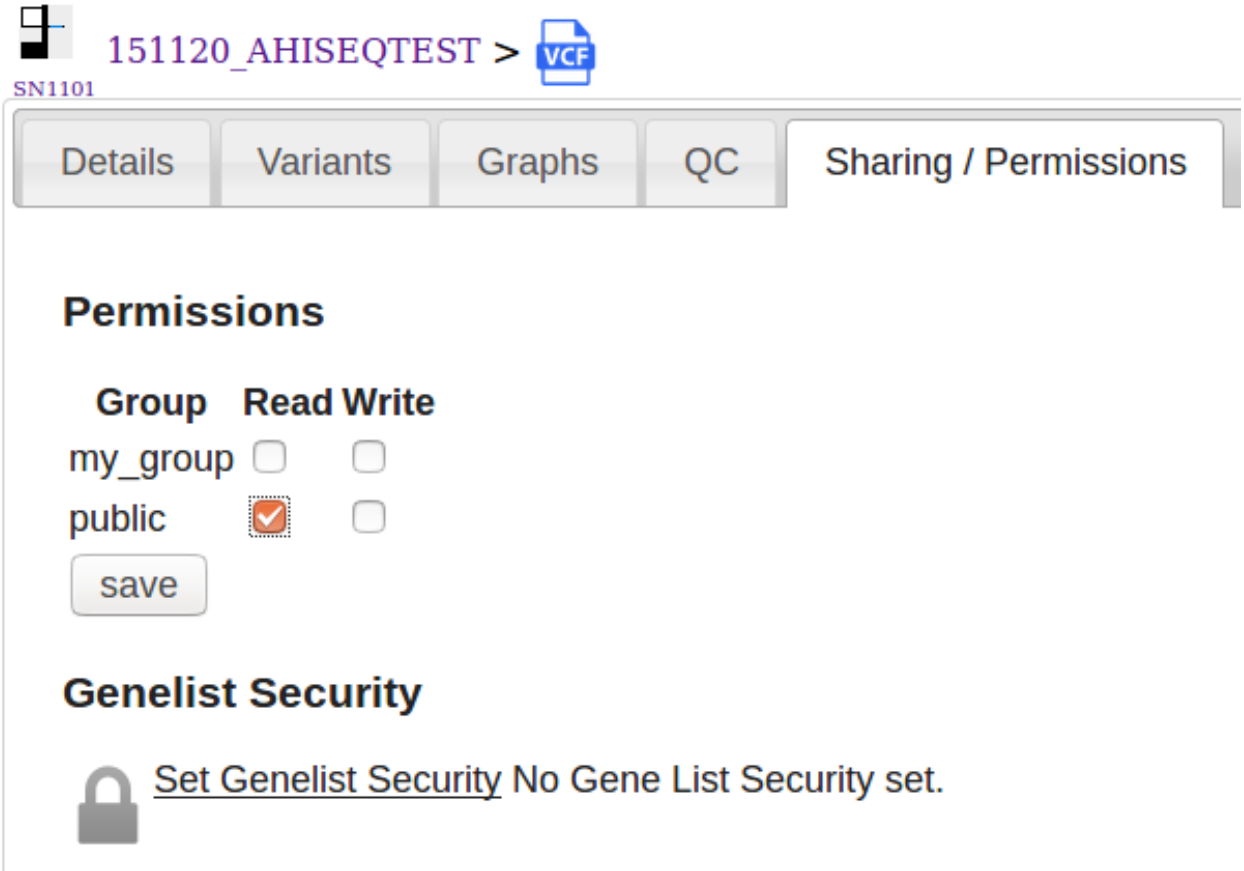
Click the link on the grid to view the file details page.

12.1 Sharing data

Users belong to groups (see *user settings*) that can share data. Ticking the **Show Group Data** checkbox will show this on a grid.

By default, you automatically share data (read-only) with your group.

To change data permissions, click the **[Data/Sharing]** tab:



logged_in_users is a special group - and means everyone who has a VariantGrid account.

12.2 Search

Enter text into the search box in the top right hand corner and press enter or click Go.

Accepted inputs:

12.2.1 HGVS

We use PyHGVS library for parsing HGVS names, which supports 'c.', 'n.' and '.p'.

CHAPTER

THIRTEEN

SOMATIC DATA

Somatic VCFs detected as somatic only (tumor minus normal) are analysed for mutational signatures

13.1 Allele Frequency

We do not import the AF value from the VCF, but instead *normalize* the data then recalculate AF to be `AD / sum(AD for all variants at locus)`

In an analysis, Sample, Cohort and Trio nodes can filter by allele frequency. For the Cohort and Trio nodes, **all** or **any** refers to requiring all samples to have allele frequency within the ranges or just one or more sample.

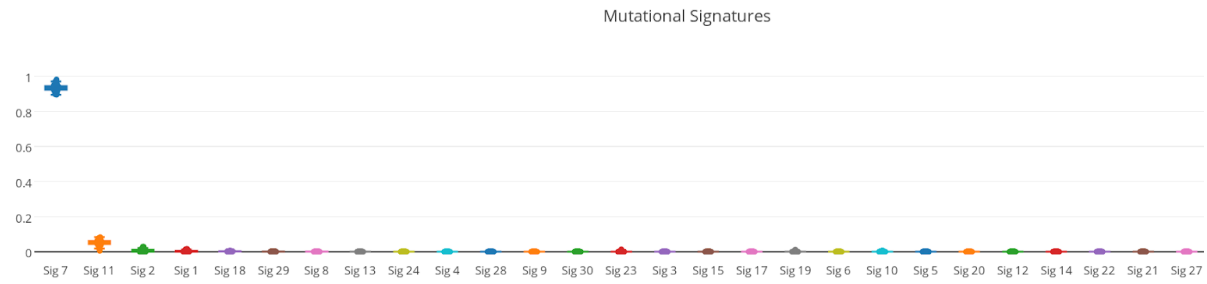
CHAPTER

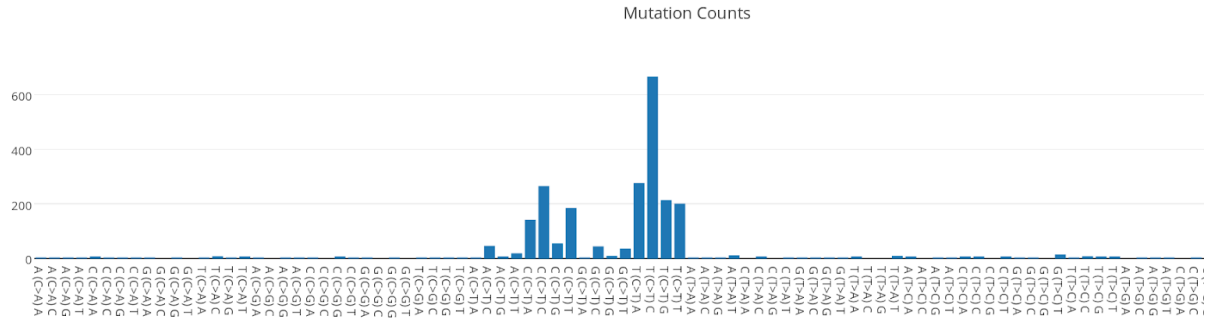
FOURTEEN

MUTATIONAL SIGNATURES

Different types of cancer can have consistent somatic variants, see Signatures of mutational processes in human cancer, Alexandrov et al 2013

Sample	161130HamishScott_somvar_combined.snvs.HC.vcf (161130HamishScott_somvar_combined.snvs.HC.vcf)
Summary	Sig 7: 93.51%, Sig 11: 5.23%
# snps	2375
Iterations	100
Sampling Fraction	0.8
Minimisation Strategy	Least Squares





Signature	Mean	Std	Cancer Types	Proposed aetiology
Sig 7	0.935142778421159	0.01660961417180388	Found predominantly in skin cancers and in cancers of the lip categorized as head and neck or oral squamous cancers.	Based on its prevalence in ultraviolet exposed areas and the similarity of the mutational pattern to that observed in experimental systems exposed to ultraviolet light Signature 7 is likely due to ultraviolet light exposure.

Mutational signatures are calculated during VCF import when the sample is detected as *somatic only*

Menu: **[data]** -> Sort samples grid by “Mutational Signature” column -> Click on entry.

Or click on the link in the “Mutational Signatures” at the bottom of the sample page.

Thanks to Paul Wang from the ACRF Cancer Genomics Facility for the code.

CHAPTER

FIFTEEN

VCF / SAMPLES

15.1 VCF import

Variants are *normalized* upon import. We only import variants, filters and genotypes (we don't use INFO as we do our own annotations)

The VCF format can vary a lot, we have tested VCFs from the following variant callers:

- GATK
- FreeBayes

Each sample is assigned a "variants type" of *Unknown*, *Germline*, *Mixed* (single sample) or *Somatic only* (tumor minus normal).

This is determined by looking at the "source" entry in the VCF header, and matching it to an entry in **VCFSource** object (setup by your administrator)

Samples with variants type of_somatic only_ are checked for mutational signatures

15.2 Multi-sample VCFs

Multi-sample VCF files combined using bam files record the genotype for all samples at each variant position.

This allows you to differentiate between reference calls and no coverage - and is extremely important for Trios so that you can make correct calls about inheritance and denovo variants

You must use bam files, to re-call the genotypes for each position.

Consider 3 VCF files:

There's no way to tell if a variant not being present in a single sample VCF is due to having the reference allele or no coverage.

Merging just the VCFs (without supplying the bams) will give the genotypes of:

If you merge them using GATK/Picard using bam files - the caller will re-examine the reads over the locus, and make the genotype call.

Thus, if both parents had reference bases, the calls would be:

And you can be confident that it is a denovo variant, rather than just lacking coverage in one of the parent samples.

CHAPTER

SIXTEEN

SEARCH

TODO

CHAPTER

SEVENTEEN

GENE PAGE

Menu: **[genes] -> [genes]** then autocomplete a gene name.

You can also enter a gene name such as “GATA2” or “RUNX1” into the search box, or click on a link in GeneGrid

If you have gene coverage data, boxplots will be shown.

CHAPTER

EIGHTEEN

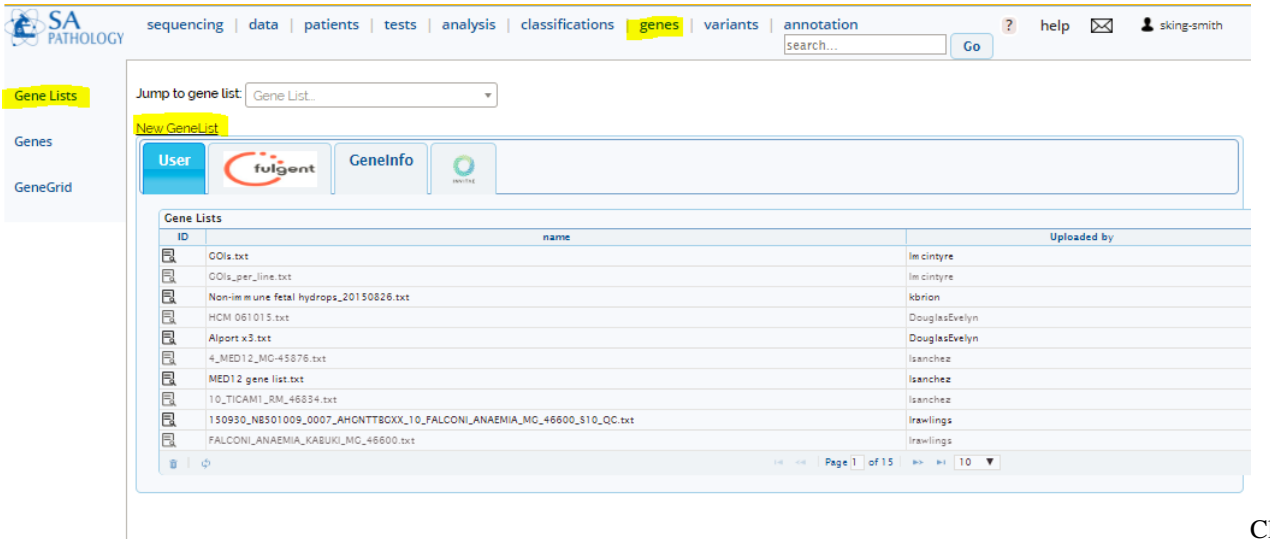
GENE LISTS

Menu: **[genes]**

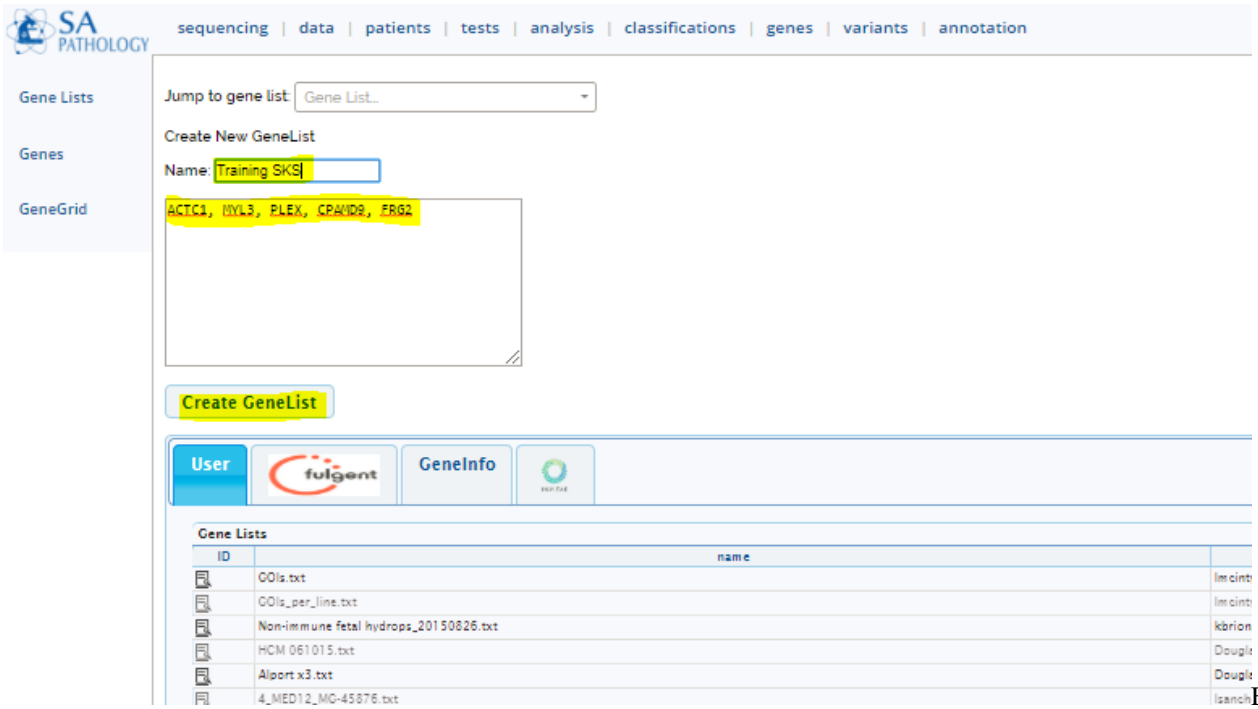
18.1 Creating Gene Lists

Ways to create a gene list include:

- Upload a text file (see *upload*)
- Create via GeneGrid
- Creating manually (see screenshot below)



Click on New GeneList

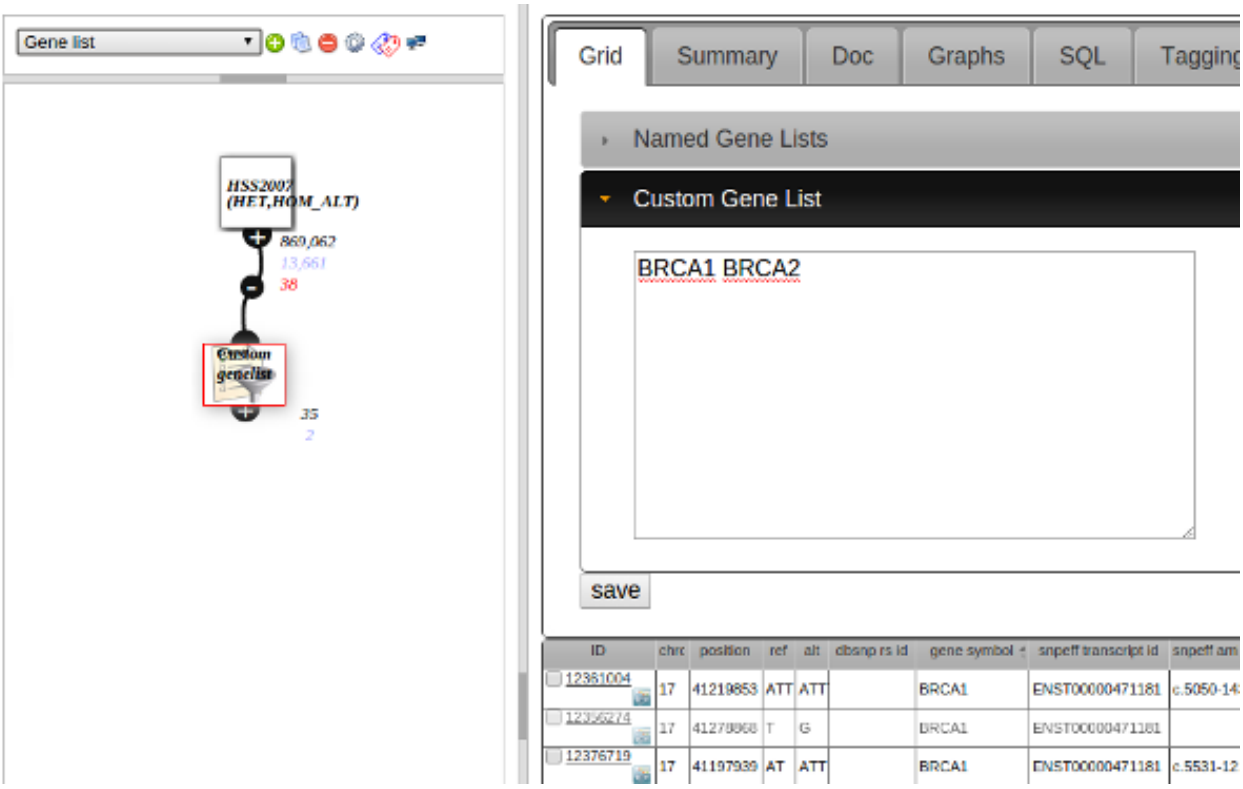


Enter name, genes and click save

18.2 Using gene lists in analyses

To quickly filter to a gene list in an *analysis*

1. Add and connect a gene list node
2. Select “Custom Gene List” in the top right node editor
3. Enter the genes into the text box and click “Save”


Gene list
HSS2007 (HET,HOM_ALT)
860,062
13,661
38
Custom gene list
35
2
Grid
Summary
Doc
Graphs
SQL
Named Gene Lists
Custom Gene List
BRCA1 BRCA2
save
ID
chrc
position
ref
alt
dbsnp rs id
gene symbol
snpeff transcript id
snpeff am
12361004
17
41219853
ATT
ATT
BRCA1
ENST00000471181
c.5050-14
12356274
17
41278868
T
G
BRCA1
ENST00000471181
12376719
17
41197939
AT
ATT
BRCA1
ENST00000471181
c.5531-12

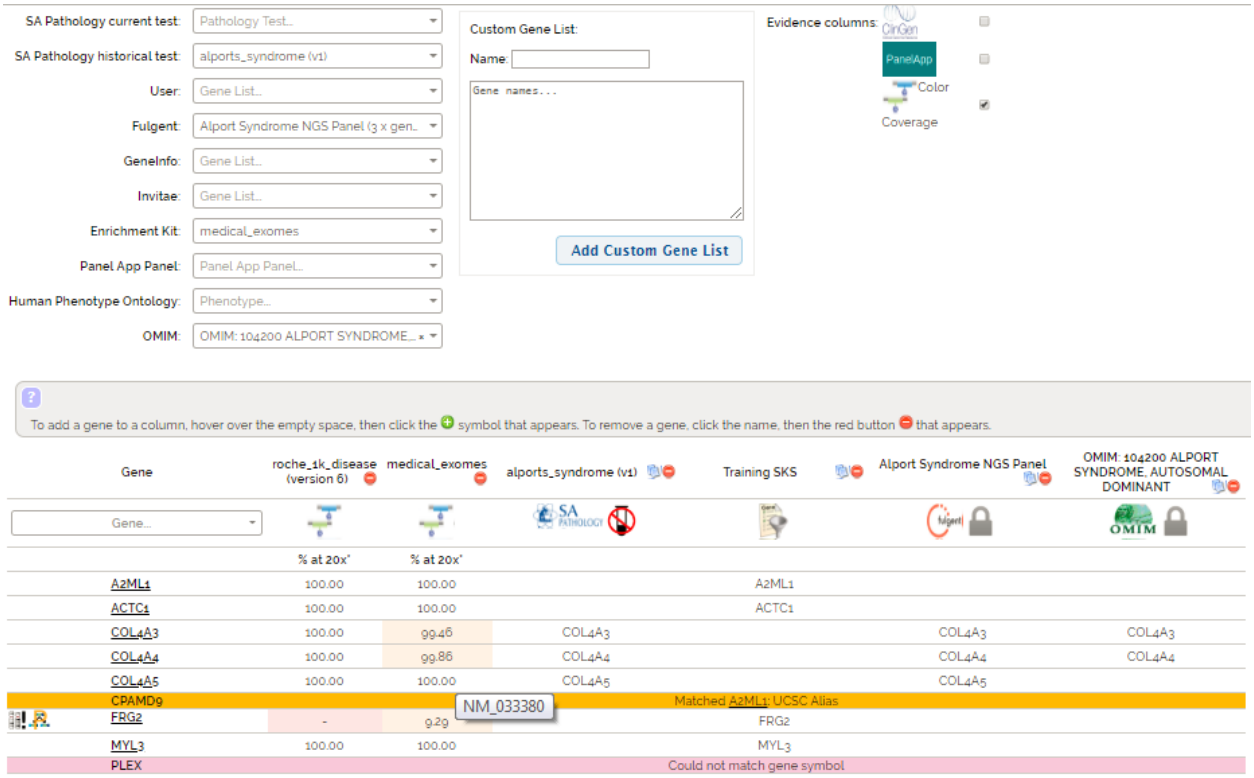
CHAPTER

NINETEEN

GENE GRID

Menu: **[genes] -> [gene grid]**

GeneGrid allows quick comparisons between gene lists and adding/removing genes from them. Genes are rows and gene lists are columns.



19.1 GeneGrid screen

You can copy/paste the URL at any time to re-create a particular comparison.

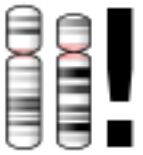
Choose lists from the top left select boxes, or manually paste in gene names into the **Custom Gene List** text entry box. Click the red delete button to remove a gene list column.

In the top right are optional evidence columns which provide information about genes.

See Gene Coverage for details on how the **% at 20x** values in the Enrichment Kit columns are calculated. Enrichment kits are automatically added when a *pathology test* that uses it is added to the grid.

19.2 Gene Info

Small icons next to gene names on the left of the grid indicate the gene has one of these attributes:

 Alternative Haplotype  Pseudogenes  Triplet repeat disorders

CHAPTER

TWENTY

GENE COVERAGE

Gene Coverage refers to how well a gene was covered by high throughput sequencing reads. This is useful to know how confident you can be about a lack of variant calls in a region.

Having gene coverage associated with a VCF sample allows you to be warned in an *analysis* when a gene in a gene list is below a threshold (default: 20x) and you may be missing some variants. The node will flash yellow, and the "genes" tab will be highlighted yellow so you can view which genes have low coverage.

Boxplots of sample coverage for genes are on the gene page

20.1 Canonical Transcripts

Many genes have multiple transcripts, but people want only one value for each gene.

This is achieved by choosing a single (representative or canonical) transcript, and use that transcripts value for the gene.

A CanonicalTranscriptCollection is a list of gene:transcript mappings imported into the system. The administrator can import different collections, linking them to EnrichmentKits and setting a system default.

20.2 Sample QC metrics

You can *upload* gene coverage files (.txt files) which use the system default canonical transcripts. You can then associate them with a *sample from a VCF*

Sample QC coverage loaded via *sequencing features* - and automatically choose transcripts based on EnrichmentKit

20.3 GeneGrid EnrichmentKit coverage

The per-gene QC metrics for an EnrichmentKit on the GeneGrid page are from *Gold Standard Runs*, using the canonical transcripts for that EnrichmentKit.

CHAPTER

TWENTYONE

PATHOLOGY TESTS

Menu: **[tests] -> [manage tests]**

Pathology Tests are curated, versioned gene lists offered as a diagnostic test. There can be multiple versions of a test.

A Pathology Test Version is a specific versions of a pathology test.

21.1 Active tests

Each pathology test has at most one currently active test - the one available for test orders.

An active test is the most recent confirmed version of a pathology test.

Active test logo

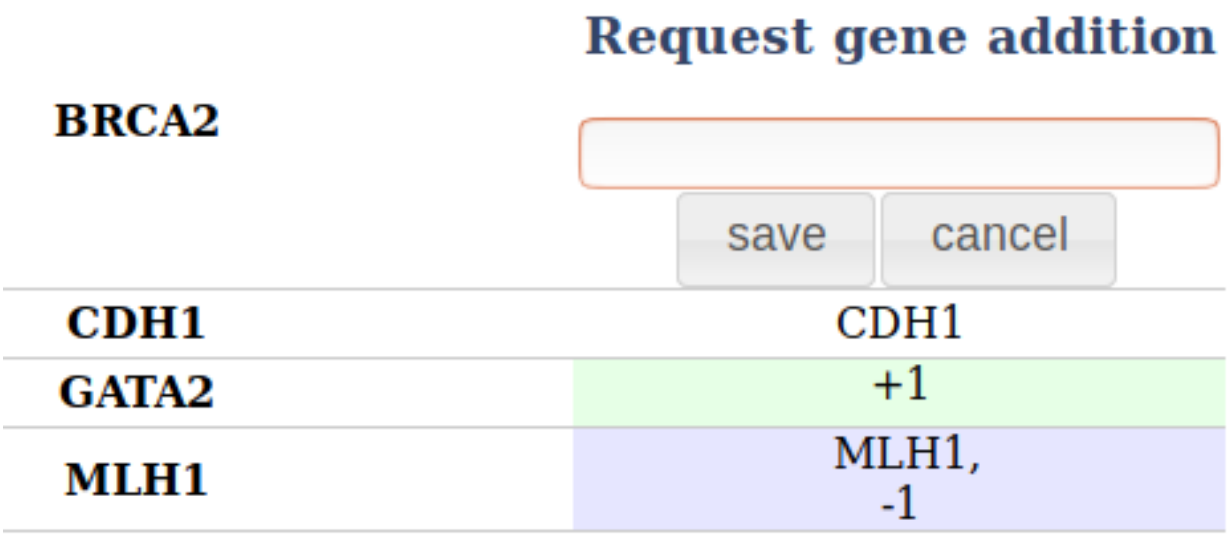
All other versions of tests

The curator confirms & adds a time-stamp by clicking the **Confirm Test** button. Once a test has been confirmed it cannot be modified, and any further changes must create a new test version.

21.2 Requesting gene changes

Only the curator can modify a test, everyone else can make modification request but these must be approved by the curator. Contact an administrator to change curator for a test.

Make gene modification requests on the *GeneGrid* page.



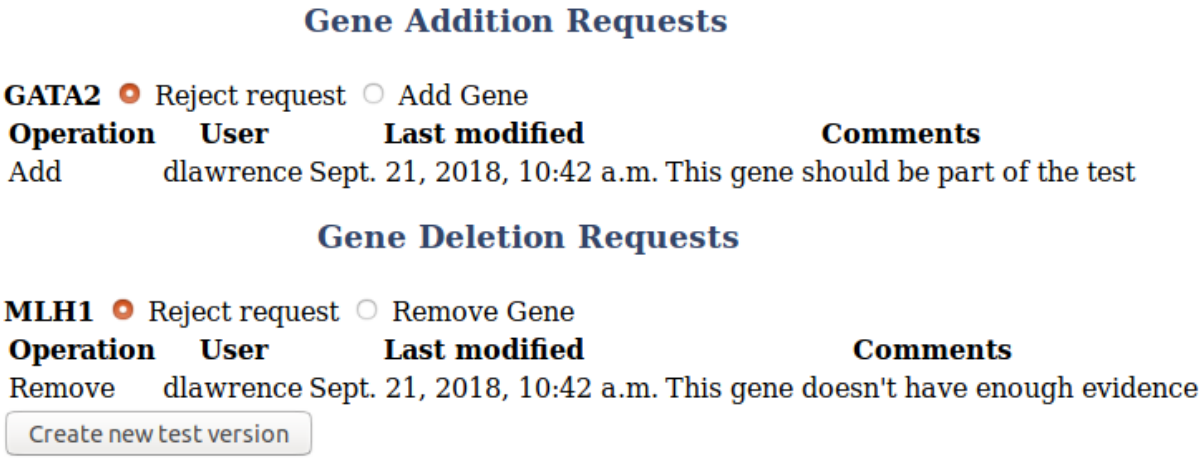
The gene symbols in the pathology test column are always what is in the test. The +/- numbers (green background for add, blue for delete) in the image above are counts of requested additions/removals for that gene.

To request a gene addition: Add genes to the *GeneGrid*, then click on an empty space where the gene should be. *To request a gene deletion:* Click on an existing gene, then the red delete symbol which appears.

In both cases a box will appear where you can enter a brief justification of the request. Only put a brief summary - please put in depth evidence such as linking a disease with a gene or adding literature on the gene page (click on the the gene name on the left column of the grid to open gene page in a new window).

21.3 Accepting gene changes

The curator can see any pending requests on the pathology test version page, where they can accept/reject them.



Any genes added will have the user, date and brief justification comment from the addition request stored on the "Modification info column" which you can see on the grid of genes for a pathology test version.

The outcomes for any processed requests can be seen by all users at the bottom of the page:

Outcome	Operation	User	Last modified	Comments
Accepted	Add	dlawrence	Sept. 21, 2018, 10:44 a.m.	This gene should be part of the test

Outcome	Operation	User	Last modified	Comments
Rejected	Remove	dlawrence	Sept. 21, 2018, 10:44 a.m.	This gene doesn't have enough evidence

CHAPTER

TWENTYTWO

TEST ORDERING

CHAPTER

TWENTYTHREE

PATIENTS

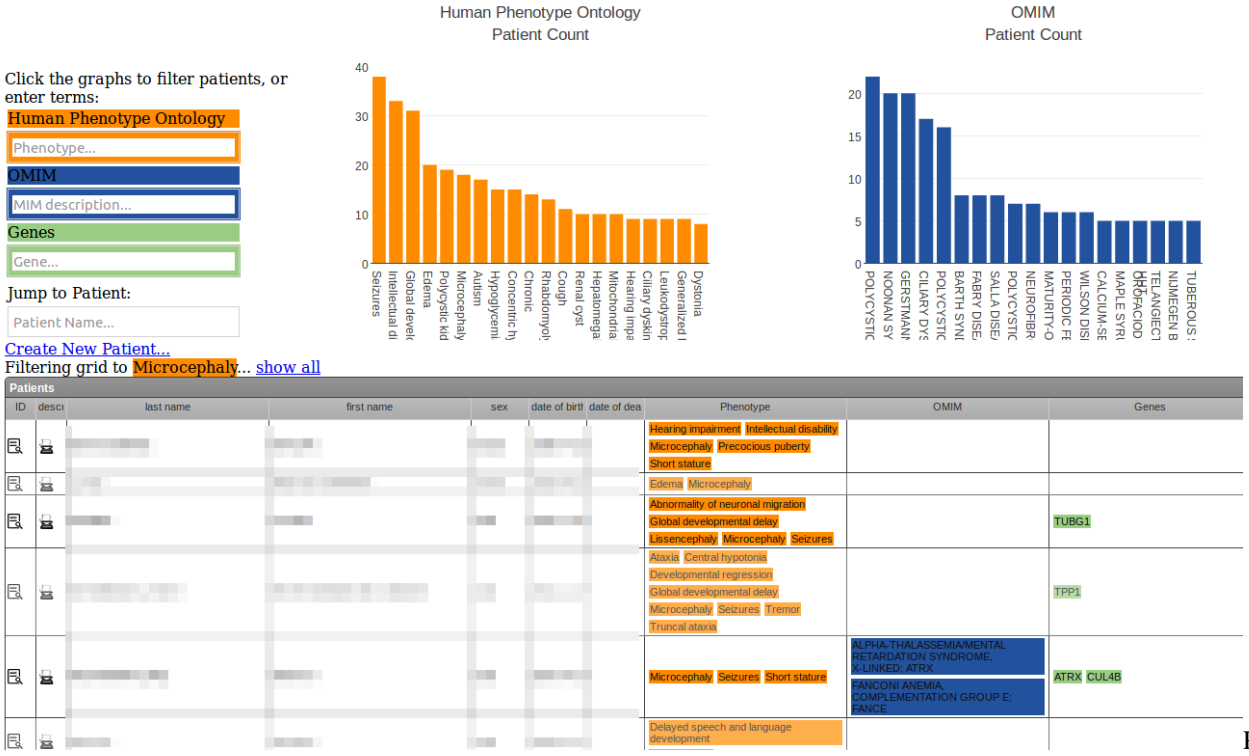
Menu: **[patients]**

Create patients to store phenotype information and link multiple samples (eg tumor/normal) together.

23.1 Searching

You can search by name, code or free text in the phenotype description.

Click the graph of phenotype terms to filter the grid to patients with that phenotype.



Patients grid filtered to microcephaly

23.2 Patient records

Import a CSV to create patients in bulk. Click the **patient record imports** link at the top of the page, then can select to download an example CSV with your samples pre-filled, so it's easy to match your patients to your existing data.

You can also create patients one at time via a form, by clicking the **Create New Patient** link just above the grid.

23.3 Other sources of patients

Patients can be created via the patholoy test ordering system.

On a private server (eg diagnostic lab intranet), patient records can be automatically created via your LIMS/Patient records system (speak to your administrator)

23.4 Other

Family Code is useful for linking together patients

The system can be configured to show/hide names, or convert birthdates to years depending on your privacy needs.

CHAPTER

TWENTYFOUR

PHENOTYPES

It is useful to store phenotypes, diseases and genes for a patient. Having this information well structured and using controlled terms is very useful as it allows us to:

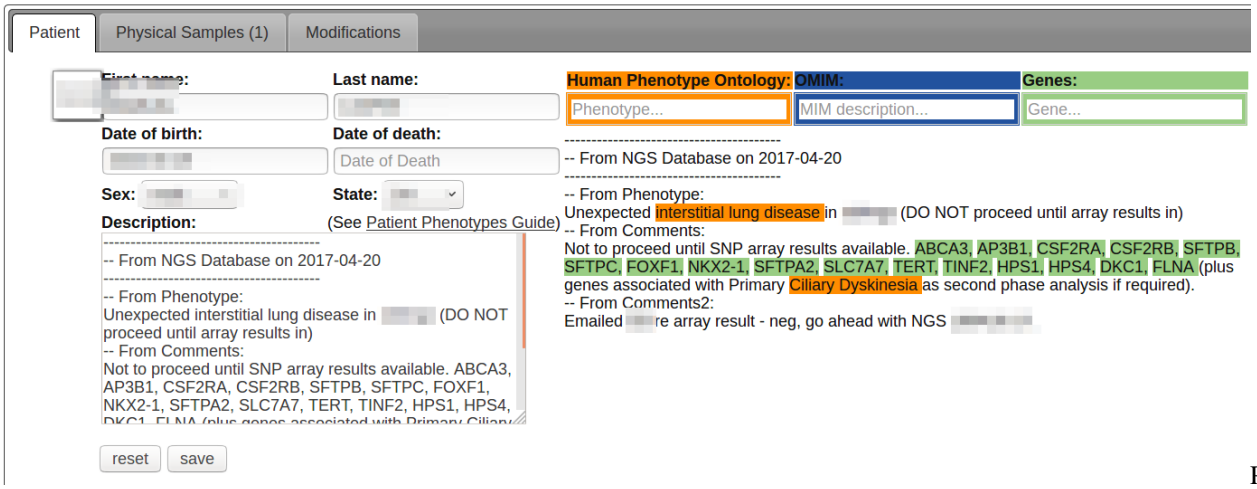
- Filter variants to genes associated with a disorder
- Know phenotypes for patients that share variants
- Perform analyses across disease cohorts (is the same variant or gene responsible for the disease or are they different?)
- Track per-disease solve rates

24.1 Assigning Terms to Patients

You can auto-complete terms in the boxes, which will be added to the bottom of the patient description.

Or, you can type plain text and we'll automatically match your words to Human Phenotype Ontology, OMIM and Gene Names.

Matched terms will be highlighted to the right of the description box.



Patients grid filtered to microcephaly

24.2 How phenotype term matching works

Everything after "–" on a line is ignored and can be used for comments.

The text is broken up into sentences based on punctuation and new lines.

The sentence is separated into words, and then sub sets of the words in order are created, and sorted largest to smallest. For instance:

```
The cat sat on the mat
cat sat on the mat
The cat sat on the
sat on the mat
cat sat on the
The cat sat on
The cat sat
on the mat
sat on the
cat sat on
the mat
cat sat
The cat
on the
sat on
mat
the
sat
cat
The
on
```

This allows us to find the biggest matches first. If a match occurs, the unmatched parts of the sentence continue to be searched until there is nothing left. If no match occurs for a sentence, we try the next smaller one.

Some filtering is done to avoid matching to common words and terms. For instance "Trio" is a gene name, but we will not match it as a gene if the sentence also contains the name of a enrichment_kit or one of the words: "exome", "WES", "father" or "mother".

Matching occurs first against Human Phenotype Ontology terms and synonyms, and OMIM terms and aliases.

If no exact match is found, we try again using mismatches - 1 mismatch (including insertions/deletions) is allowed for two or more words.

For single words, we only allow mismatches if the word is more than 5 letters long and made entirely of letters (ie no digits or symbols).

Single words are then matched (exact with no mismatches) to gene names.

Sometimes there will be multiple matches, eg "PKD1" will map to both the OMIM term PKD1 (POLYCYSTIC KIDNEY DISEASE 1) and the gene PKD1. This is usually what people want as the gene is associated with the disorder.

CHAPTER

TWENTYFIVE

COHORTS

Menu: **[patients] -> [cohorts]**

A cohort is a collection of samples, which you can analyse as a group. A multi-sample VCF automatically becomes a cohort, but you can create your own to organise your own samples.

25.1 Create a new cohort

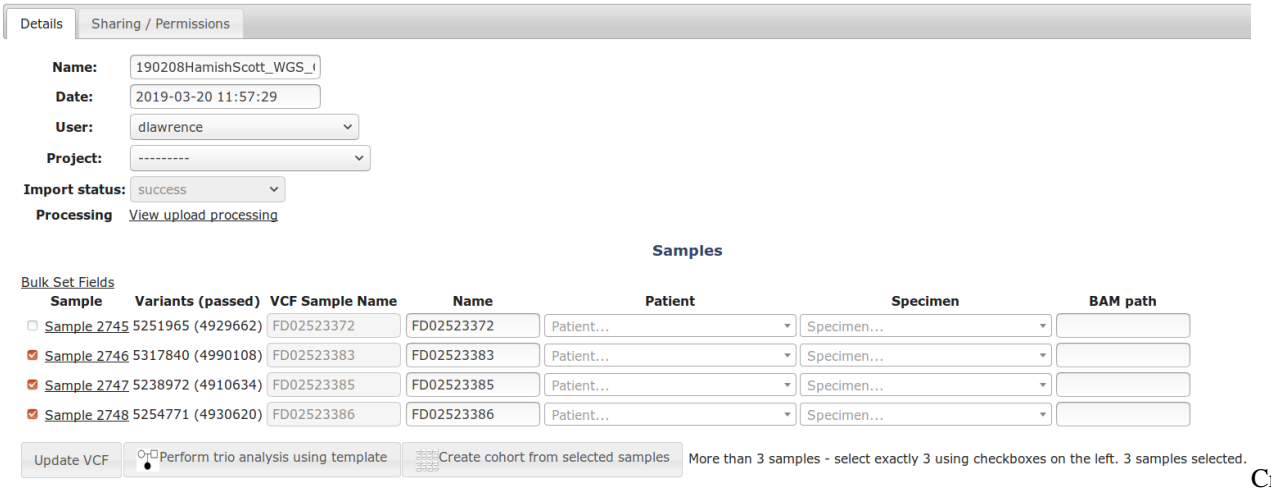
From the cohort page, enter the name of a cohort and click the **Create** button.

This opens the Add/Remove samples tab. Add samples to your cohort by auto-completing sample names in the Enter to add box, or filter the grid, select the checkbox to the left of a sample, and click the green arrow to add, or red button to delete.

Once you have finished adding/removing samples, click save. This processes the cohort so it can be used in analyses.

25.2 Create from a larger cohort

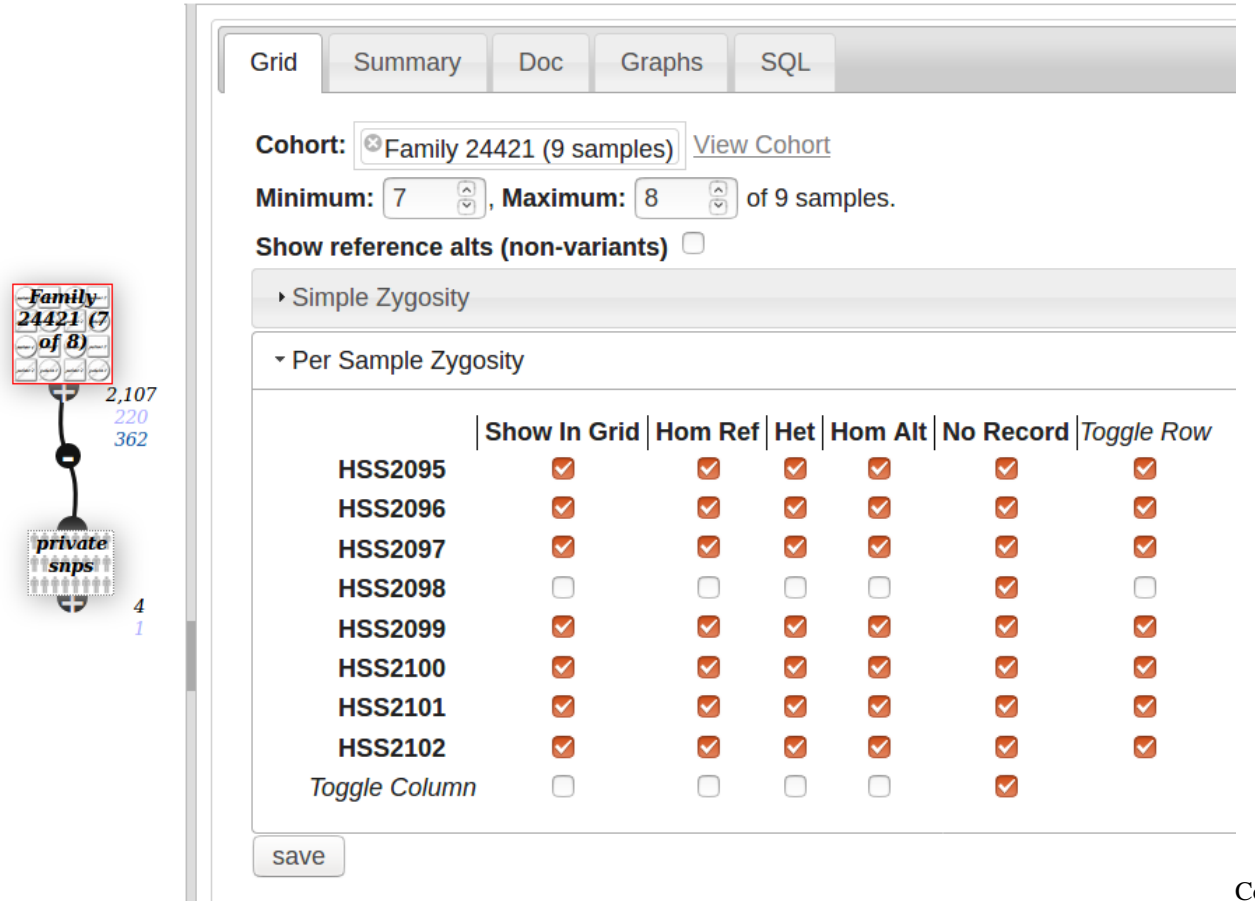
You can create a smaller cohort from a larger one. Select at least 2 samples then click the **[Create cohort from selected samples]** button. Selecting exactly 3 samples allows you to create a Trio which allows for simpler analyses.



Creating a sub-cohort

25.3 Cohort Analyses

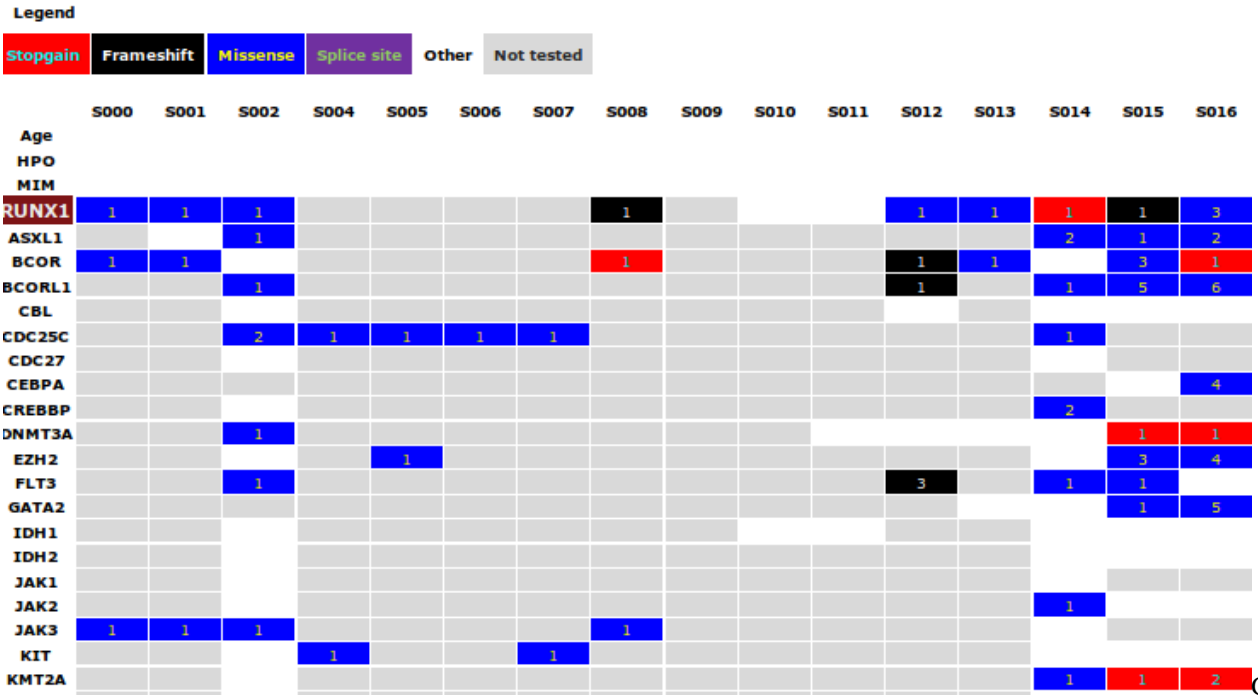
Use the Cohort Node to filter by counts within the cohort (eg in 7 out of 8 of the samples) or zygosity. (see screenshot below).



Cohort Node filtering by zygosity

Quickly create an analysis using the cohort by clicking "Create new analysis for cohort" on the details tab of the cohort page.

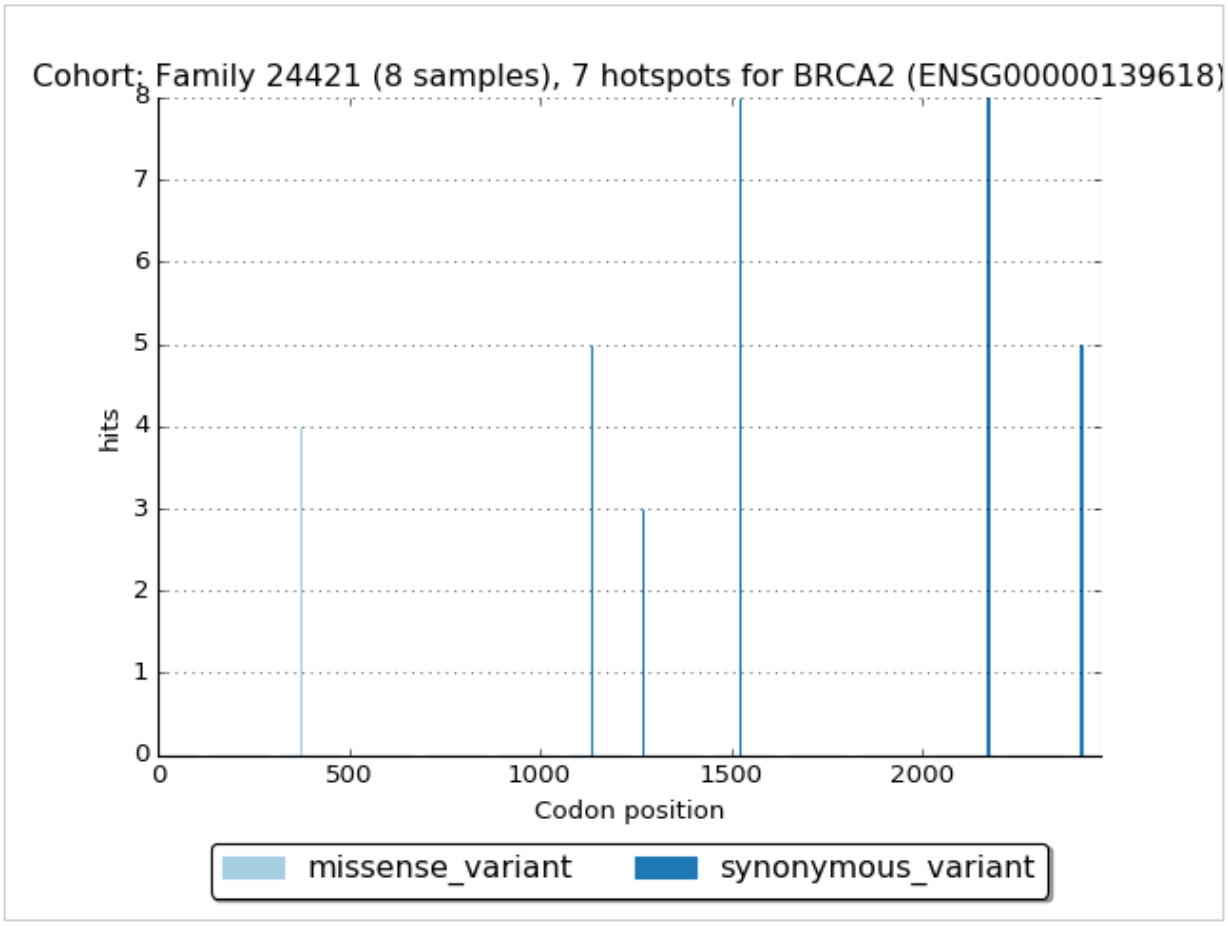
There are some other analyses you can perform from the cohort/VCF page, eg:



Gene/Sample Matrix

Cohort: Family 24421

Gene BRCA2 (ENSG00000139618) View Gene



Cohort Hotspots graph

CHAPTER

TWENTYSIX

TRIOS

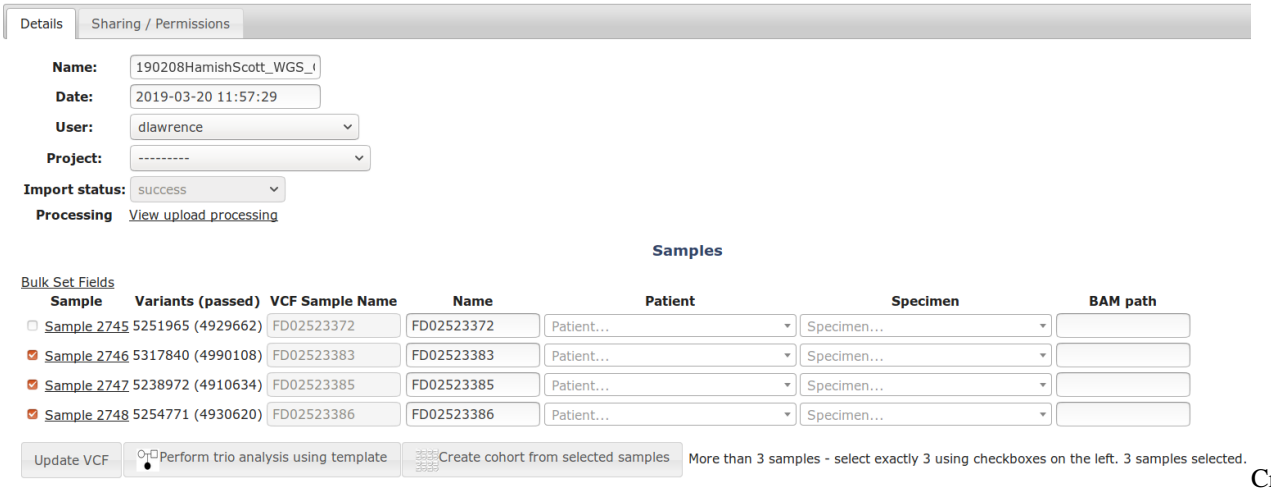
Menu: **[patient] -> [trios]**

A trio is a collection of 3 samples (mother/father/proband) which are frequently analysed together in high throughput sequencing, as they have a number of standard analyses.

26.1 Creating a trio

It is far better to upload a trio within the same *multi-sample VCF*. If not, you must first create a cohort containing the 3 samples/

View the VCF or cohort, select exactly 3 samples then click the **[Perform Trio Analysis using template]** button.



Creating a Trio

The Trio wizard will now open, showing the 3 samples and patient / phenotype info. Assign samples (1 each to mother/father/proband) and check mother or father affected if they also have the disorder.

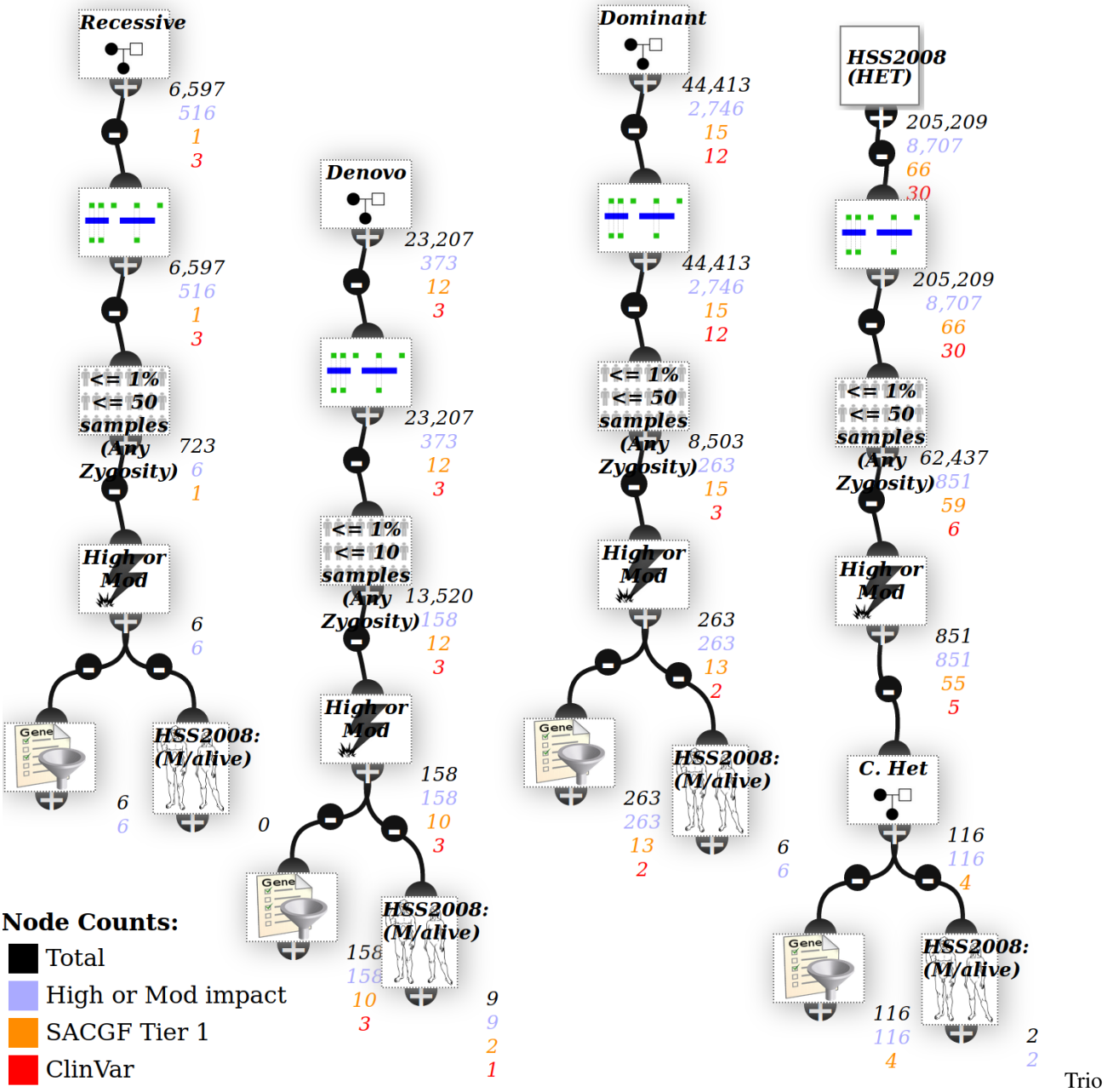
26.2 Digital karyomapping

By checking a trio's zygosity, it's possible to perform a number of relatedness calculations, see *karyomapping*.

A genome-wide count is automatically performed, and a summary provided on the trio page - this is useful for checking for sample mix-ups.

26.3 Trio inheritance analysis

An analysis is created using different inheritance models (see below). If either parent is affected it will also use an autosomal dominant inheritance model.



Trio inheritance analysis

The phenotype at the bottom uses the proband patient phenotypes, and sample gene lists.

26.3.1 Require Zygosity Calls

By default, the filters are strict and require zygosity calls in all patients - for instance the recessive inheritance model requires a variant to be HOM in proband and HET in both parents.

However that may be overly strict - one parent may have low coverage, with no variants recorded at that locus.

Click on an Trio node to open the editor - unchecking the **require zygosity calls** box is less strict and allow for variants that are missing due to low coverage.

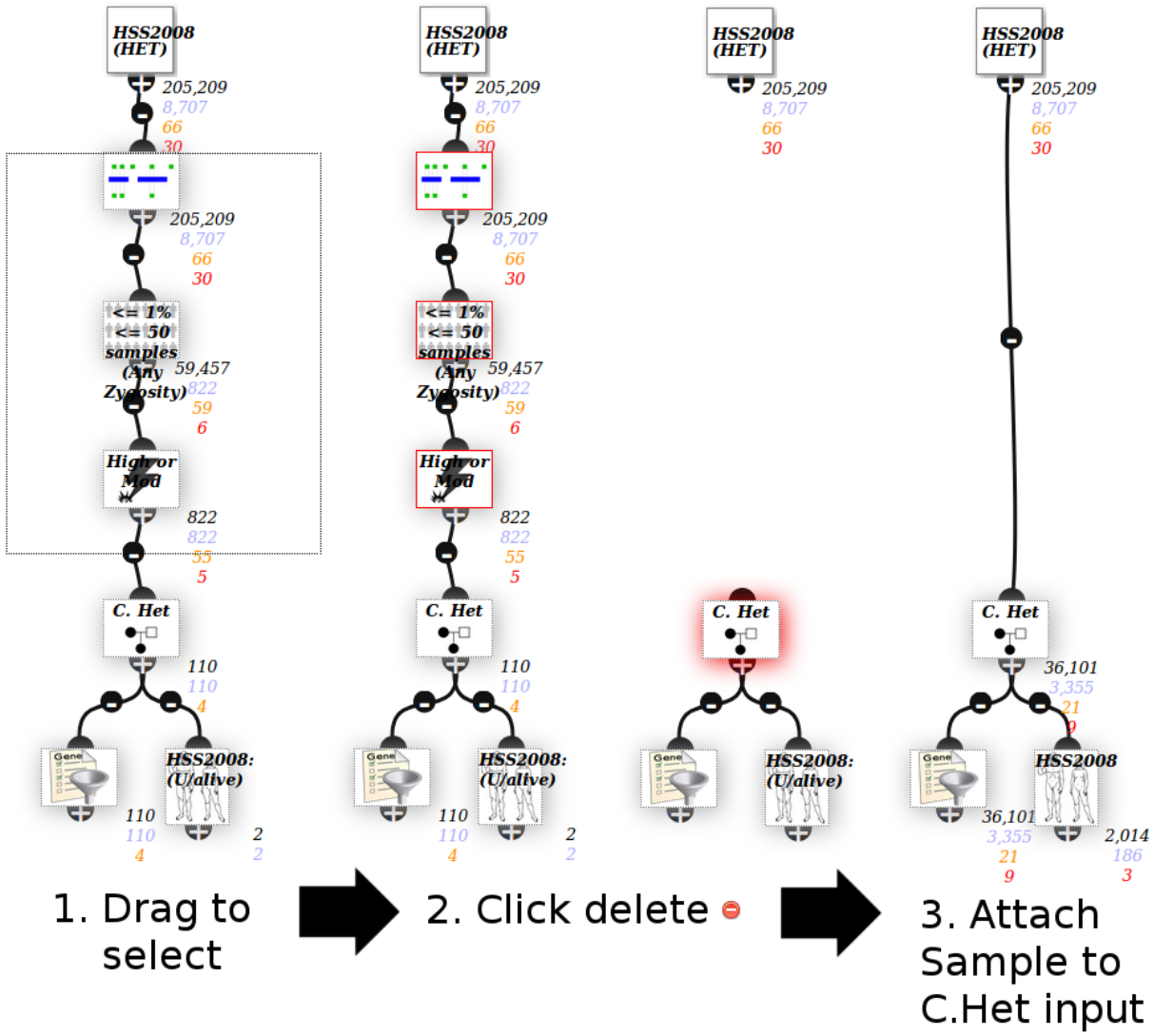
26.3.2 Compound Het filter

Compount heterozygous means 2 variants in the same gene from different parents.

The C. Het node in the bottom right of the screenshot above is a filter node - ie it has another node connected to the top, while the other inheritance models do not.

This is because you probably don't want every gene with >=2 variants, but rather only >=2 damaging/rare ones. Adjust the filters above the C.Het node to adjust this.

Modify the analysis as per instructions below to filter to all of them.

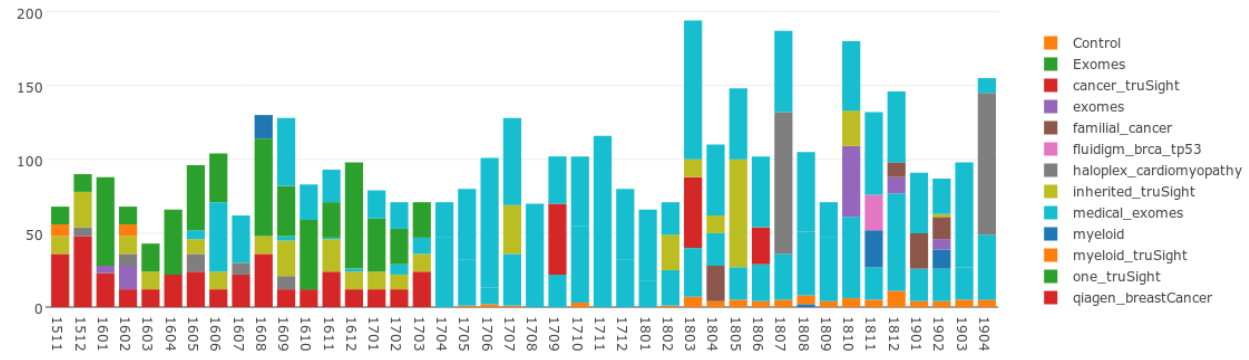

HSS2008 (HET)
205,209
8,707
66
30
205,209
8,707
66
30
<= 1% <= 50 samples (Any Zygosity)
59,457
822
59
6
High or Mod
822
822
55
5
C. Het
110
110
4
HSS2008: (U/alive)
110
110
4
2
2
1. Drag to select
2. Click delete
3. Attach Sample to C.Het input
36,101
3,355
21
9
HSS2008
2,014
186
3

CHAPTER

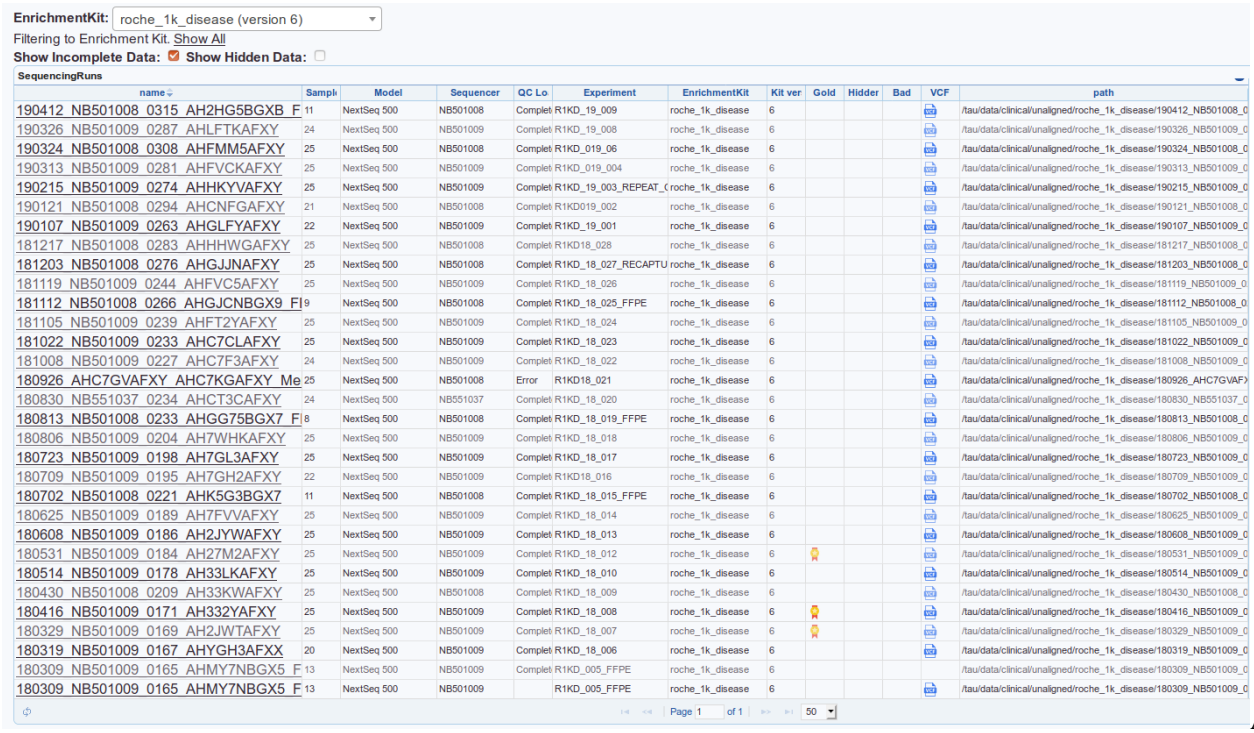
TWENTYSEVEN

SEQUENCING RUNS

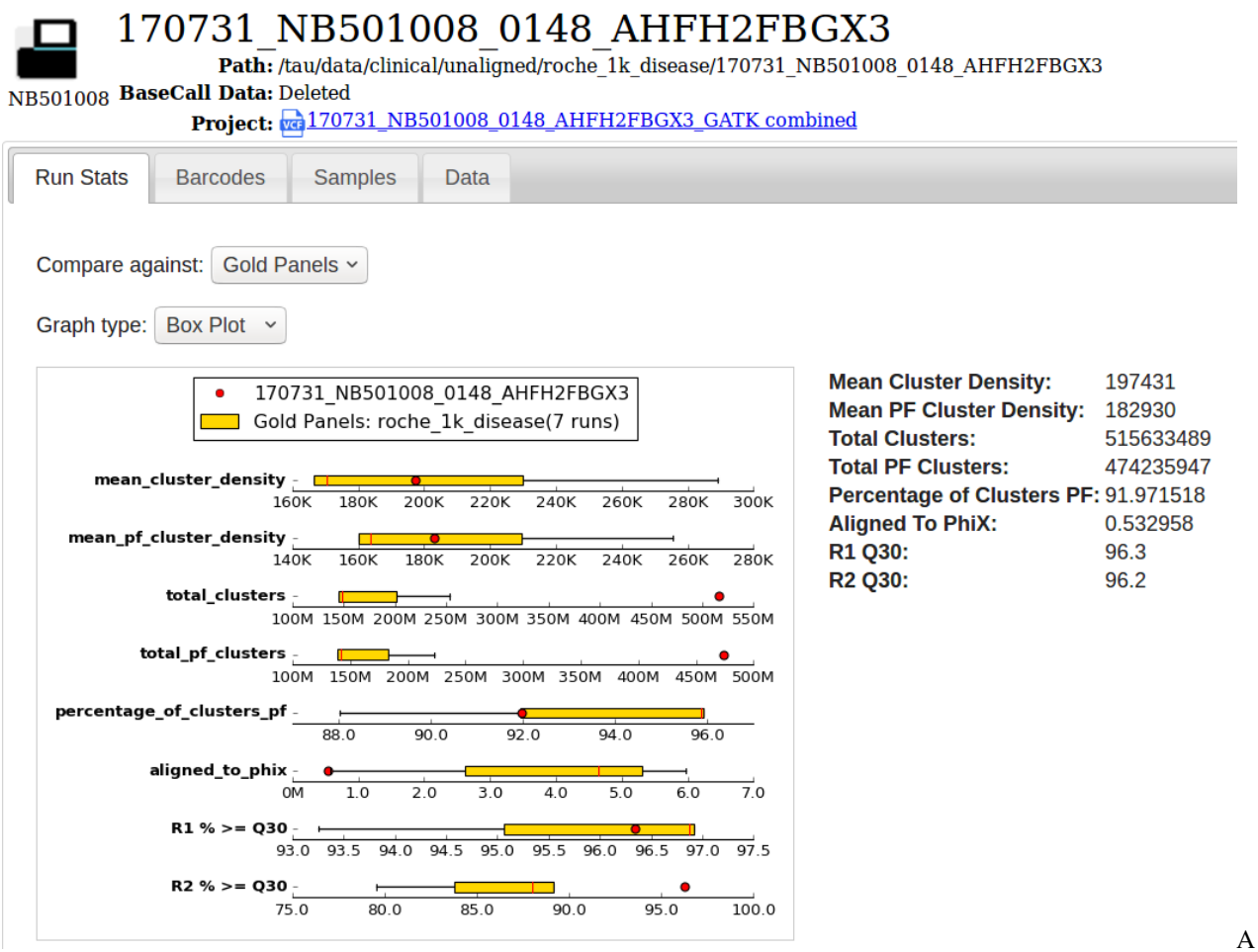
When VariantGrid has access to a network drive (eg a diagnostic lab intranet) it can scan disks for sequencing runs to collect QC metrics, gene coverage and automatically load VCFs.



Sequencing Samples over time



Automatically loaded sequencing runs + VCFs

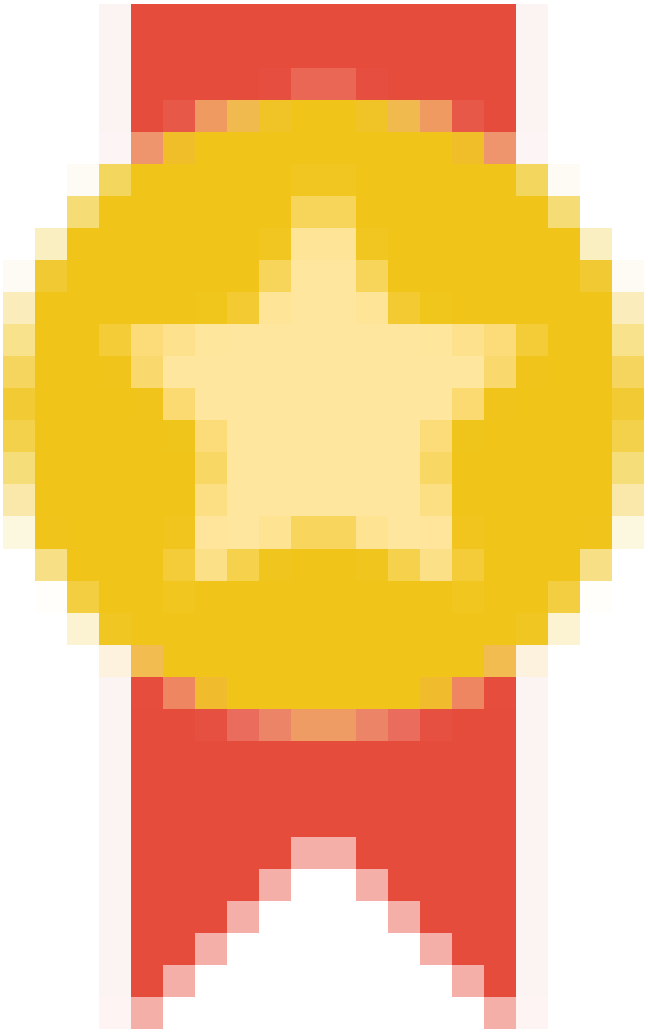


A Sequencing Run

We collect Sequencing QC metrics and display them with interactive graphs. Collecting data over time allows us to see how this run compares to other runs over time (or vs *gold standard runs*).

27.1 Gold Standard Runs

The administrator can mark a sequencing run as "Gold Standard" - which means it has passed validation / is of sufficient quality to be used as a benchmark for other runs.

Gold standard runs have an icon ()
on the sequencing run grid.

Gold runs for an enrichment kit are used:

- In boxplots on QC metrics pages for a sequencing run or other sample QC graphs.
- To calculate average *gene coverage* on the *GeneGrid* page.

27.2 Finding sequencing data

Sequencing Runs are found by searching for the file 'RTAComplete.txt' on the server disks. You can ignore flow cells by putting a file ".variantgrid_skip_flowcell" in the directory.

CHAPTER

TWENTYEIGHT

USER SETTINGS

Lab Password

28.1 Customise columns

CHAPTER

TWENTYNINE

CUSTOMISE COLUMNS

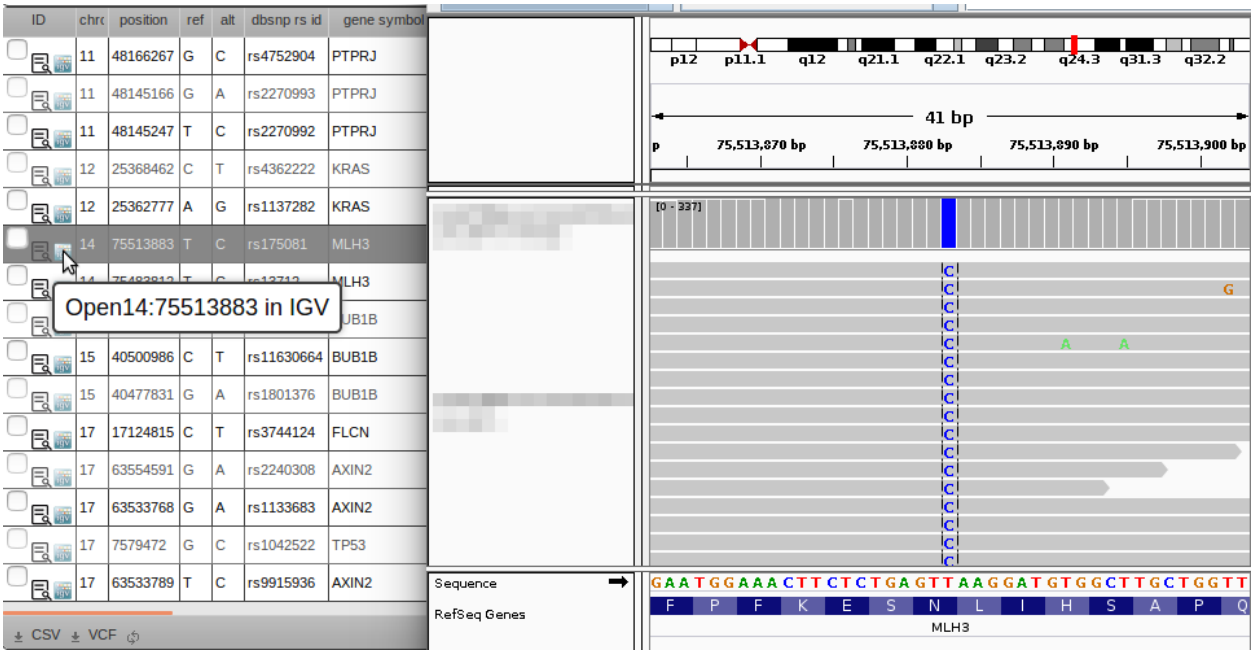
You can customise grid columns on the **Customise Columns** ([user]->[customise columns]) page.

CHAPTER

THIRTY

IGV INTEGRATION

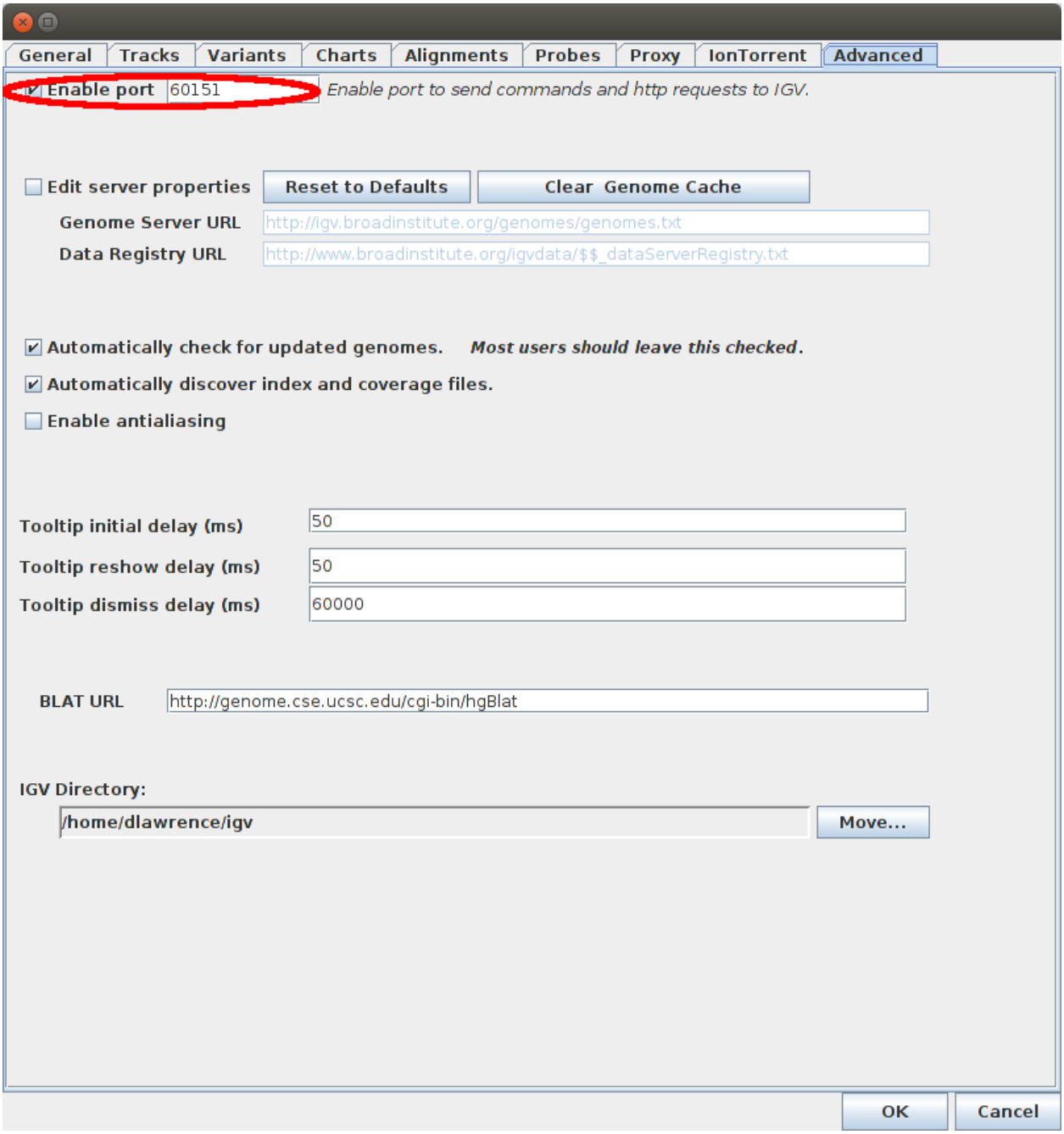
Click the  IGV link to automatically jump to your variants + BAM files in IGV.



30.1 IGV Configuration

IGV needs to be running, and have the Enable Port option ticked.

To check this open preferences in the IGV menu: **[View] -> [Preferences] -> [Advanced] Tab**.



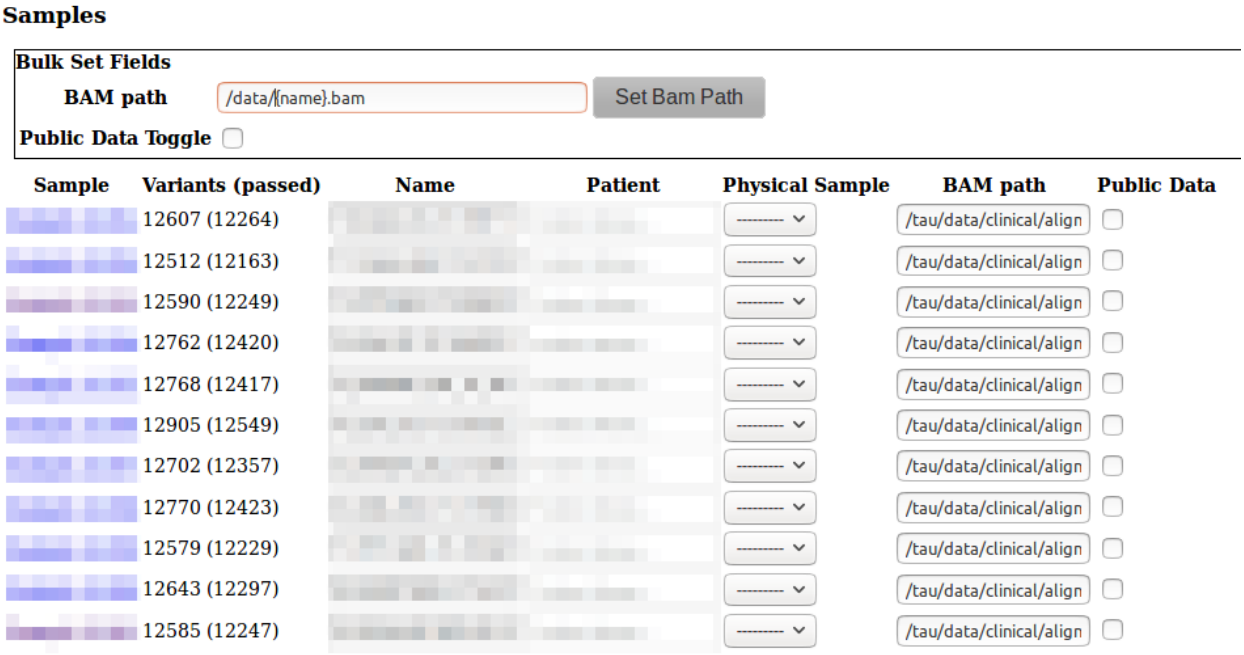
30.2 VariantGrid Configuration

If the value of the IGV port is different from **60151** (default), you need to change the IGV Port option in your User Settings page.

Clicking the IGV link (IGV link) will jump to the locus, and show BAM files associated with input samples (Sample or Cohort ancestors). These are the same samples that have their zygosities/allele depth shown on the grid.

Each sample has a bam file path entry. If your samples were automatically loaded from a server, this is probably already set. Otherwise you can change it on the Sample or VCF (VCF) page.

You can set all the samples in a VCF file at once in the vcf page, click Bulk Set Fields to set all samples according to a pattern based on the sample name.



30.3 Network drives and File Servers

Many labs access data via servers, or network shares. These can be different on different computers.

It is recommended that you set bam file path to be the location on the server, so that it is consistent between users.

Different data access methods on different computers can then be managed by having users change their configuration on the IGV Integration page.

CHAPTER

THIRTYONE

VARIANT CLASSIFICATIONS

31.1 Creating Classifications

- From an analysis (see *analysis classification workflow*)
- From the *variant details page*
- Via API (See Shariant API docs)

31.2 Autopopulation

When you create a classification from inside the system, a number of fields are auto-populated from annotation and sample information.

Variants created from the external API are not auto-populated with values from annotation.

31.3 Editing

See the Classification Form.

31.4 Configuring Fields

An administrator can add/remove EvidenceKeys which are used to create fields.

They can also hide visible fields on a per-lab basis.

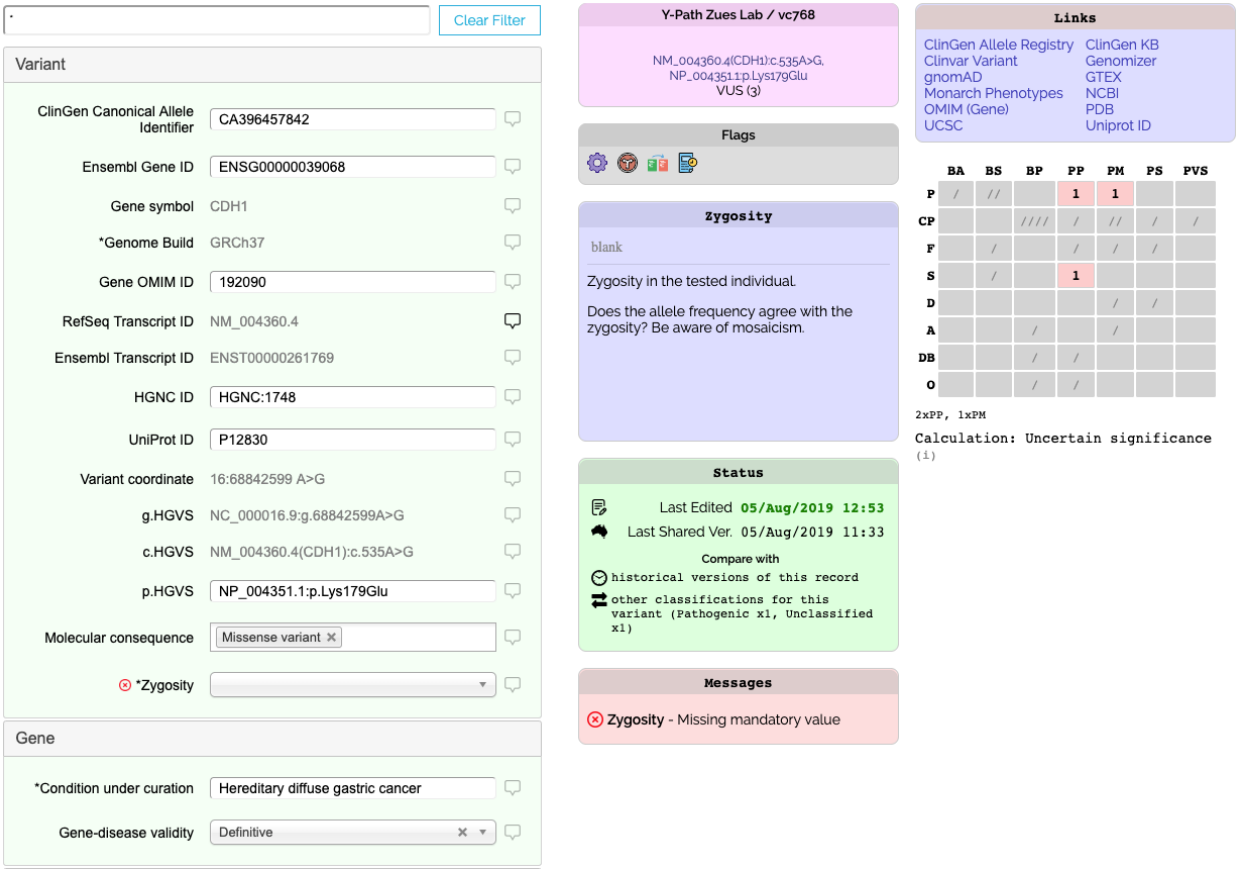
CHAPTER

THIRTYTWO

VARIANT CLASSIFICATION FORM

The Classification Web Form can be used to create and edit classifications directly within VariantGrid.

32.1 View



To quickly see all fields that have values for a classification, enter "*" into the filter box at the top of the classification. To see all possible fields, enter "**" in the filter box. To find an individual field, start typing the label of the field into the filter e.g. "gnomad".

32.2 Identify Errors

A record might not be shared as there are outstanding validation errors. In the Messages box on the form it will list any errors. If possible fix those errors in your curation system and then they should be fixed on the next sync.

32.3 Change History / Diff

Each version of a record published in VariantGrid is recorded, by clicking on "Compare historical versions of this record".

If there are other classifications for the same variant, there will be a link to compare them there too.

32.4 ACMG Guidelines

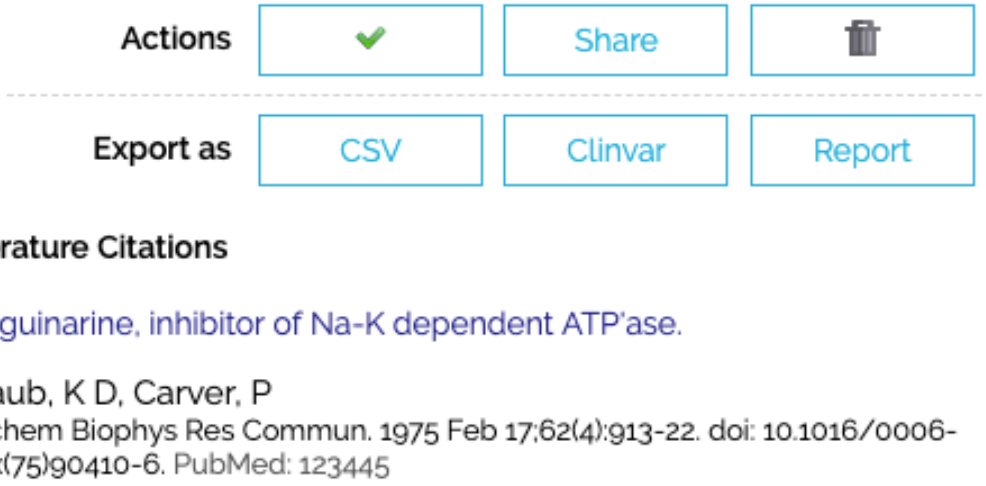
The classification form has fields for the ACMG Guidelines, e.g. PM4, BA1 - the meaning of each is given in the help. See Guidelines

VariantGrid displays a grid of ACMG fields with each row being a category of data, and each column representing the strength of evidence for benign or pathogenic.

- The number of met criteria for a given box will be shown as a number.
- Explicitly unmet criteria will show as "/"s.
- Criteria not yet marked as met or unmet will show as "?"s.

The various values will be plugged into the ACMG formulae and a recommended overall clinical significance will be displayed. This calculated value has no affect on any of the data, the user is still able to set the overall clinical significance to whatever (hopefully justifiable) value they like.

32.5 Actions



At the bottom of the form there will be a list of action buttons.

The Tick icon re-submits the classification at its current change level. For any manual changes to be seen, this button will need to be ticked.

Next to it is a Share button that allows you to increase the scope of who can see the classification. Important, increasing the Share level is not un-doable. The share levels are

- Just your lab
- Anyone within your organisation (if your organisation has multiple labs)
- All Shariant Users
- 3rd Party Databases (this will allow us to upload the record to Clinvar at a later date)

32.6 Delete / Withdraw

If the classification has only been shared at the lab or organisation level, you are able to perform a hard delete on the record. If it has been shared, instead you have the option to "withdraw". This will remove the record from most listings and search results, but will not remove it from any Discordance Reports that it had been involved in (it will no longer be a part of discordance calculations).

When a record has been withdrawn it can be unwithdrawn by clicking the same button (it should look like a rubbish bin with a raised lid now).

32.7 Export

You can also export the single record as CSV, a preview of the Clinvar format or as a report. (The report does require that your lab has a report template pre-configured.)

32.8 Literature Citations

Any PMID references in the form of PMID:123456 from anywhere within the classification will be summed together and listed at the bottom of the classification.

CHAPTER

THIRTYTHREE

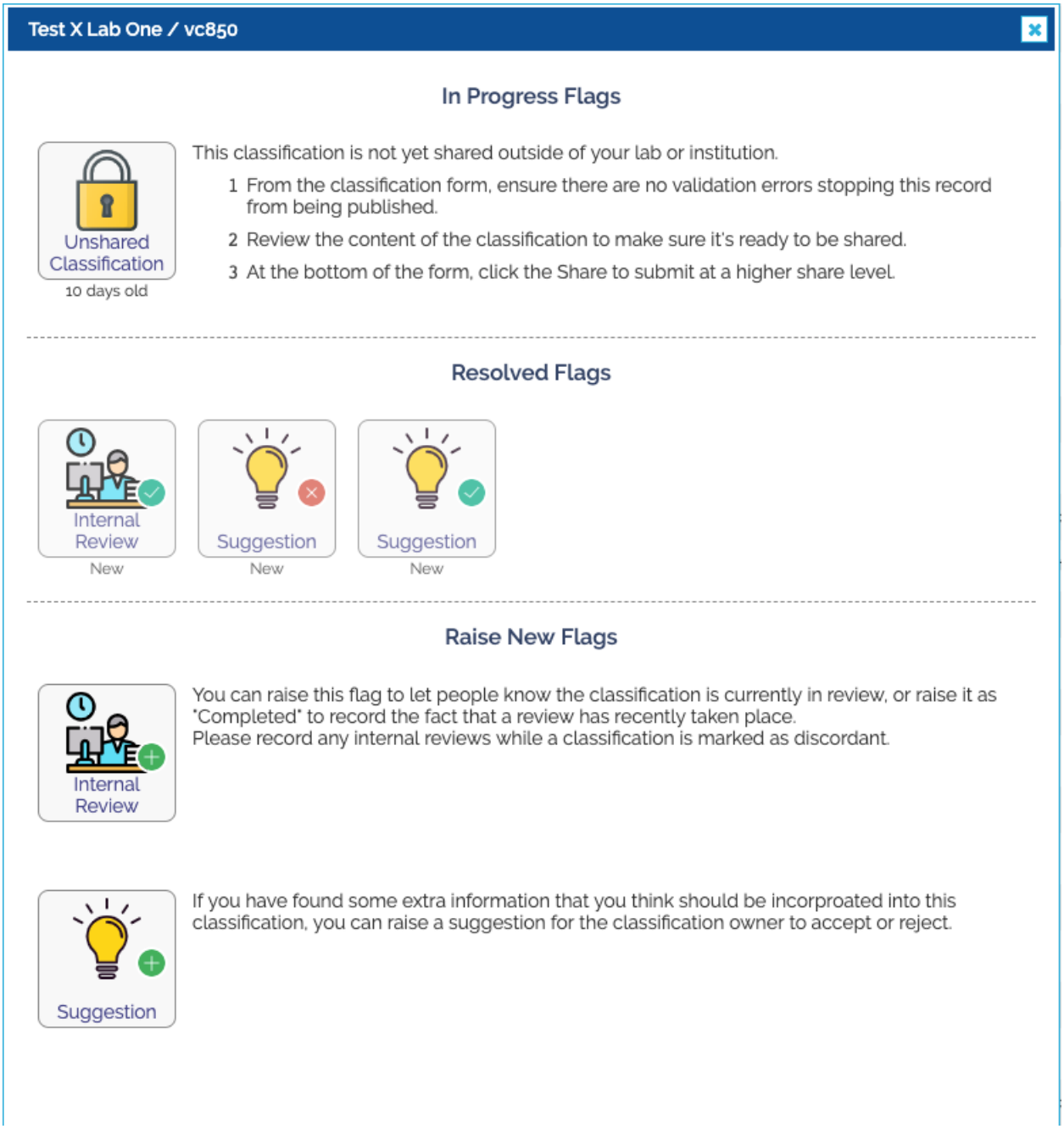
CLASSIFICATION FLAGS

Each classification flag indiciates that there is an action that needs to be performed against the classification.

Many of the flags will be automatically raised by Shariant, though some of them you will be able to open yourself.

To look at the details of a specific open flag, simply click on it to be taken to the flag dialog.

33.1 Flag Dialog



From the flag dialog you can view summaries about what flags are currently open, see a list of flags that have been resolved as well as raise new ones. Note that only important flags still show up when closed, e.g. suggestions and internal reviews and a few others.

In the provided screenshot we can see we have an open flag asking us to share the classification, a completed internal review, an accepted suggestion and a rejected suggestion, as well as the buttons to create new internal reviews and suggestions.

You can visit the details of an open flag, or a closed one by clicking on the icon.

From the details page of an open flag, depending on the type of flag, you can add a comment and potentially change the status of a flag.

You can raise a new flag by clicking on one of the icons near the bottom with a plus button.

(The kinds of actions you can take on flags will depend on if you're looking at a classification from your lab or another lab.)

See below for flags and how to solve them:

33.2 Flag Types

33.2.1  Discordance

This classification is in discordance with one or more classifications.

1. Ensure that you have completed an internal review of your lab's classification recently (within the last 12 months is recommended). If not, raise the internal review flag and complete an internal review of your lab's classification.
2. Review any outstanding suggestions against your lab's classification.
3. View the other classifications in the discordance report and view the evidence differing between multiple records via the diff page. If appropriate, raise suggestions against other lab classifications.
4. This Discordance flag will automatically be closed when concordance is reached.

This is discussed in the Classification Discordance page.

33.2.2  Internal Review

This classification is marked as currently being internally reviewed.

1. Once the internal review is complete, ensure you update the classification in your curation system.
2. Mark the internal review as Completed.

This is discussed in the Classification Discordance page.

33.2.3  Matching Variant

This variant has not been seen in this system previously. It should be linked to a variant given time.

33.2.4  Matching Variant Failed

We were unable to normalise the variant provided based on the c.hgvs and genome build values.

1. Please contact Shariant support for help in resolving this.

33.2.5  Outstand Edits

Edits have been made to this classification that are not included in a published version.

1. From the classification form, ensure there are no validation errors stopping this record from being published.
2. At the bottom of the form, click the tick to submit the outstanding changes.

33.2.6  Significance Changed

This classification has changed it's clinical significance compared to a previously published version.

1. Set the status of this flag to reflect the primary reason behind the change in classification.
2. Please also add a comment providing some context.

This is discussed in more detail on the Classification Discordance page.

33.2.7  Suggestion

Someone has raised suggestion(s) against this classification.

1. Review the contents of each suggestion.
2. If appropriate, make changes in your curation system and mark the suggestion as Complete.
3. If you decline the suggestion, mark it as Rejected.

33.2.8  Unshared Classification

This classification is not yet shared outside of your lab or institution.

1. From the classification form, ensure there are no validation errors stopping this record from being published.
2. Review the content of the classification to make sure it's ready to be shared.
3. At the bottom of the form, click the Share to submit at a higher share level.

33.2.9  Withdrawn

This classification has been marked as withdrawn. It will be hidden from almost all searches and exports.

1. If the classification is not of high enough quality or in error, you may leave it as "withdrawn" indefinately.
2. If you wish to un-withdraw the classification, click the open bin icon in actions from the variant classification form. (Note you can't open a Withdrawn flag, but you can Withdraw/Unwithdraw from the classification form)

CHAPTER

THIRTYFOUR

VARIANT CLASSIFICATION REPORT

34.1 Running the report

To generate the report from a classification, open the classification and scroll to the bottom. You will see a button called "Report". Click on it and you will then be able to copy & paste the report contents into a document.

34.2 Configuring the report

The report can only be configured by admin users. Each "organisation" within variantgrid uses its own report. To edit it go to the admin view, Organisations, (your organisation), and then edit the Classification report template.

The template is run using Django template and produces HTML

34.3 Values available for the report

34.3.1 Evidence Keys

All the fields in the classification are exposed here, see the Evidence Keys admin for a list of possible values, e.g. zygosity, mechanism_of_disease, mode_of_inheritance. In addition you can also suffix `_raw` or `_note` e.g.

```
The raw value for Mode of Inheritance is {{ mode_of_inheritance_raw }} and the note␣
↪for it is {{ mode_of_inheritance_note }}
{% if mode_of_inheritance_raw == 'x_linked' %}
Special case for X Linked
{% endif %}
```

Typically you'll only want to refer to the `_raw` value if you're doing some logic for a specific drop down value. If you ommit the `_raw` then you will get the human friendly label for the value which might subtly change in the future.

34.3.2 p.hgvs

You can reference the full `p_hgvs` or breakdown

```
full p.hgvs = {{ p_hgvs }}<br/>
p amino acid from = {{ p_hgvs_aa_from }}<br/>
p hgvs codon = {{ p_hgvs_codon }}<br/>
p hgvs amino acid to = {{ p_hgvs_aa_to }}
```

34.3.3 c.hgvs

You can reference the full `c_hgvs` or breakdown

```
full c.hgvs = {{ c_hgvs }}<br/>
c hgvs transcript = {{ c_hgvs_transcript }} or {{refseq_transcript_id}}<br/>
c hgvs gene symbole = {{ c_hgvs_gene_symbol }} or {{ gene_symbole }}<br/>
c hgvs short = c.{{ c_hgvs_short }} (this is the value in c_hgvs after "c.")
```

34.3.4 Evidence weights

A summary of the strength of ACMG critieria met can be accessed with

```
Evidence weights = {{ evidence_weights }}
```

34.3.5 Citations

PMIDs put anywhere in the classification can be accessed, and then specific attributes of those citations can be referenced. `citations` is an array that you must loop through, e.g.

```
{% for cit in citations %}
        <tr>
                <td>{{ cit.source }}</td>
                <td>{{ cit.citation_id }}</td>
                <td>{{ cit.citation_link }}</td>
                <td>{{ cit.journal }}</td>
                <td>{{ cit.journal_short }}</td>
                <td>{{ cit.title }}</td>
                <td>{{ cit.year }}</td>
                <td>{{ cit.authors }}</td>
                <td>{{ cit.authors_short }}</td>
                <td>{{ cit.abstract }}</td>
        </tr>
{% endfor %}
```

The example here is in a table but you can display it however you'd like, e.g.

```
{% for cit in citations %}
{{ cit.source }}:{{ cit.citation_id }}
{% endfor %}
```

Which would give you PMID:12334 PMID:4555 etc

CHAPTER

THIRTYFIVE

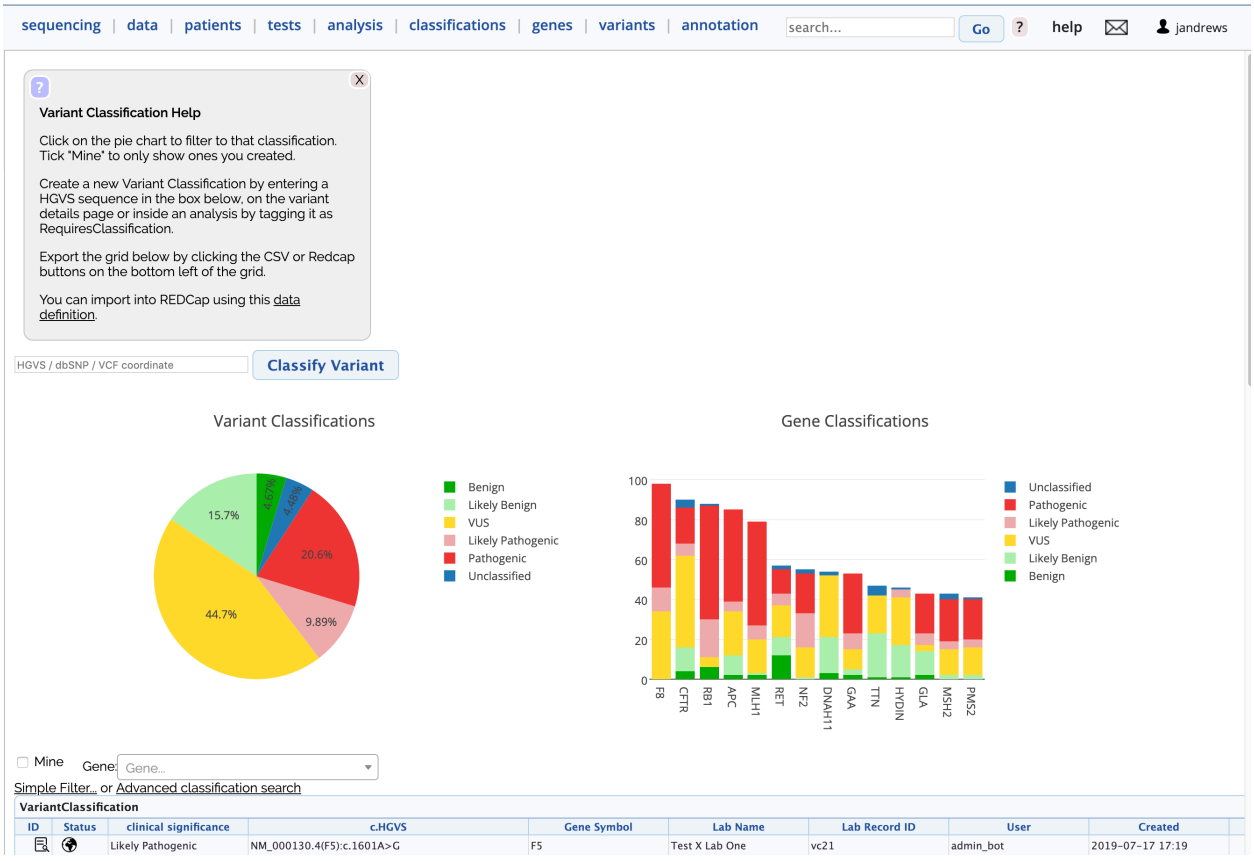
VARIANT CLASSIFICATION REDCAP

Variantgrid supports the exporting of Variant Classification data into REDCap files. Note that this is currently the full extent of REDCap integration with Variantgrid, there is no support for importing REDCap records or exporting any other kinds of records in a REDCap format.

There are two parts to the REDCap export.

35.1 REDCap Definition

The data definition is available by opening the page help on the classification page.



The definition is dynamically generated from the variant classification evidence key configuration. We do our best to ensure that changes to evidence keys are backwards compatible for REDCap definitions.

The definition is laid out in such a way that up to 10 records can be grouped together in one record e.g. `vc_zygosity_1, vc_zygosity_2, vc_zygosity_3 up to vc_zygosity_10` This is so that variants for the same patient can be consolidated.

Note that the REDCap definition is primarily used as a read only representation of the data, doing large edits of data in REDCap is not recommended.

35.2 REDCap Rows

Important: Variant Classifications will ONLY be exported if `REDCap Record ID` has a value. All rows that do not have a value for `REDCap Record ID` will be ignored in the export.

At the bottom of the classification table there will be a CSV and REDCap download button. Clicking the REDCap download will download records that are:

- Available in the current filter (if the results are split over multiple pages all will be downloaded). For example if you filter to show "Mine" the records in the download have to belong to you.
- Have a value for `REDCap Record ID`.

Records that have the same `REDCap Record ID`, regardless of any other factors, will be grouped together as described earlier, re `vc_zygosity_1, vc_zygosity_2` etc

35.3 Technical Specifics

This means while single drop down fields work as you'd expect, multi-drop downs produce text that's harder to report on.

The evidence key definitions for selects have an explicit index for each drop down option. If adding more options (regardless of insertion order) a new index should be assigned and existing options should retain their index. This is to help keep newer REDCap definitions compatible with older REDCap records.

CHAPTER

THIRTYSIX

VARIANT NORMALIZATION

CHAPTER

THIRTYSEVEN

INDICES AND TABLES

- genindex
- modindex
- search