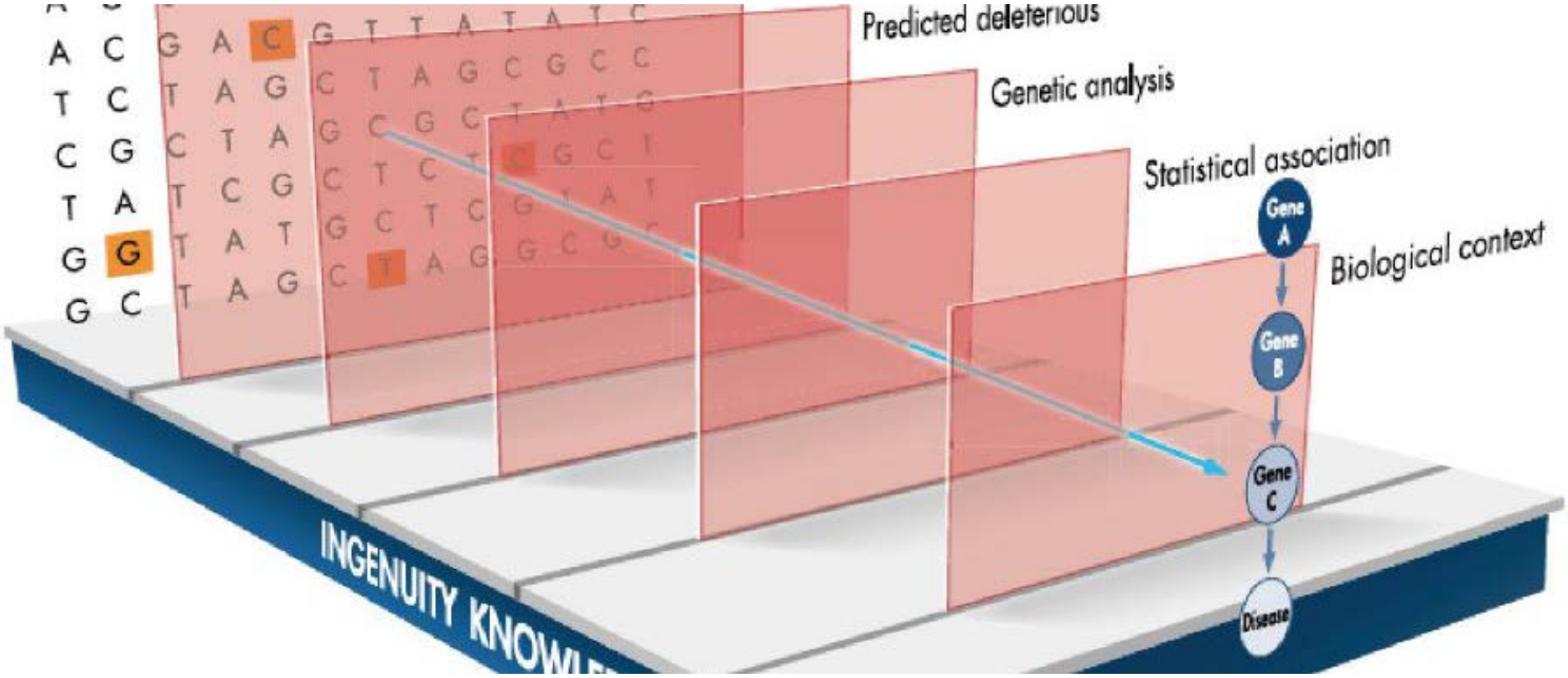


Hands-on Workshop

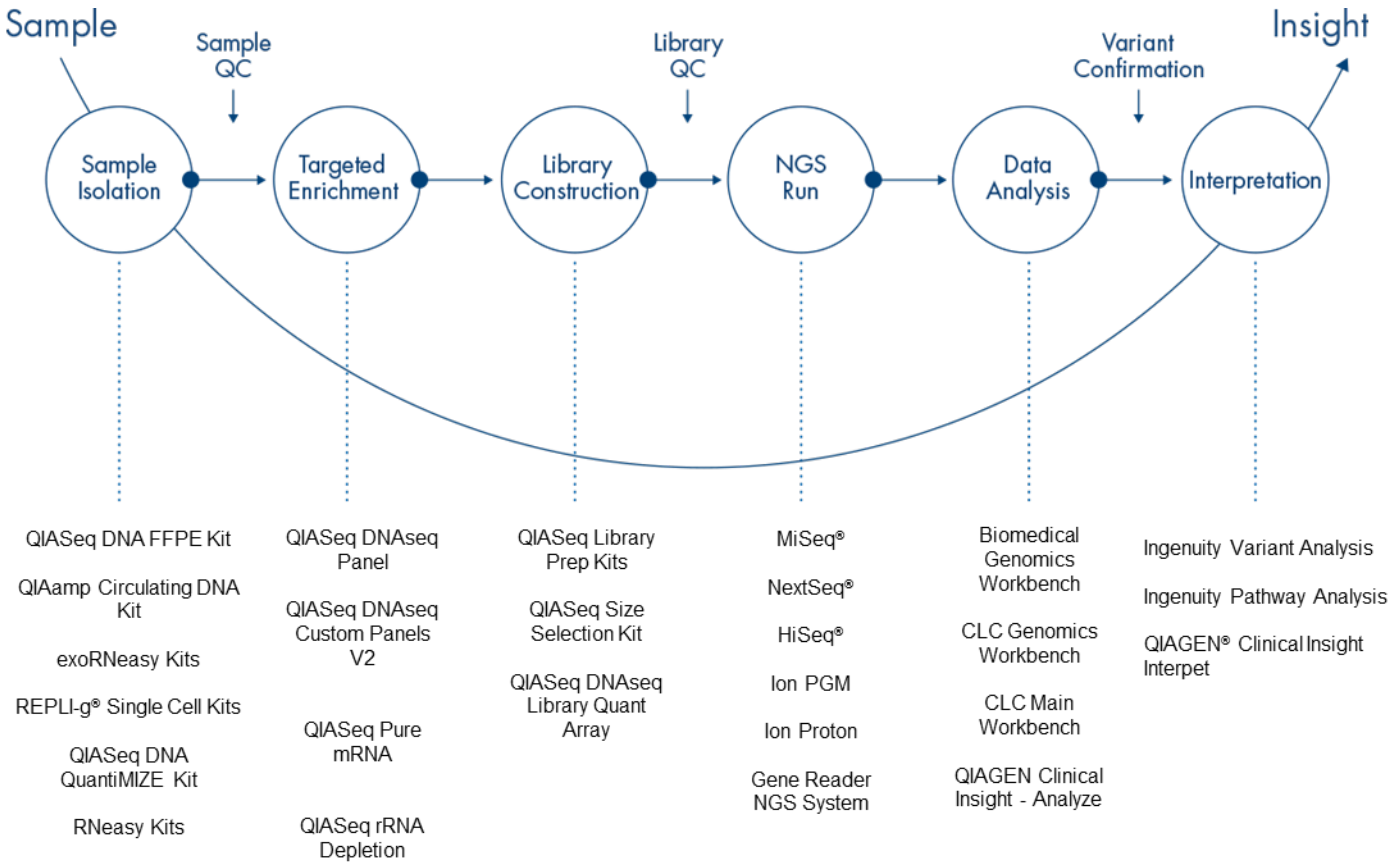
Clinical Genomics - Interpretation and Reporting with QIAGEN Bioinformatics

Ruth Burton PhD. – Clinical Applications Specialist (ruth.burton@qiagen.com)

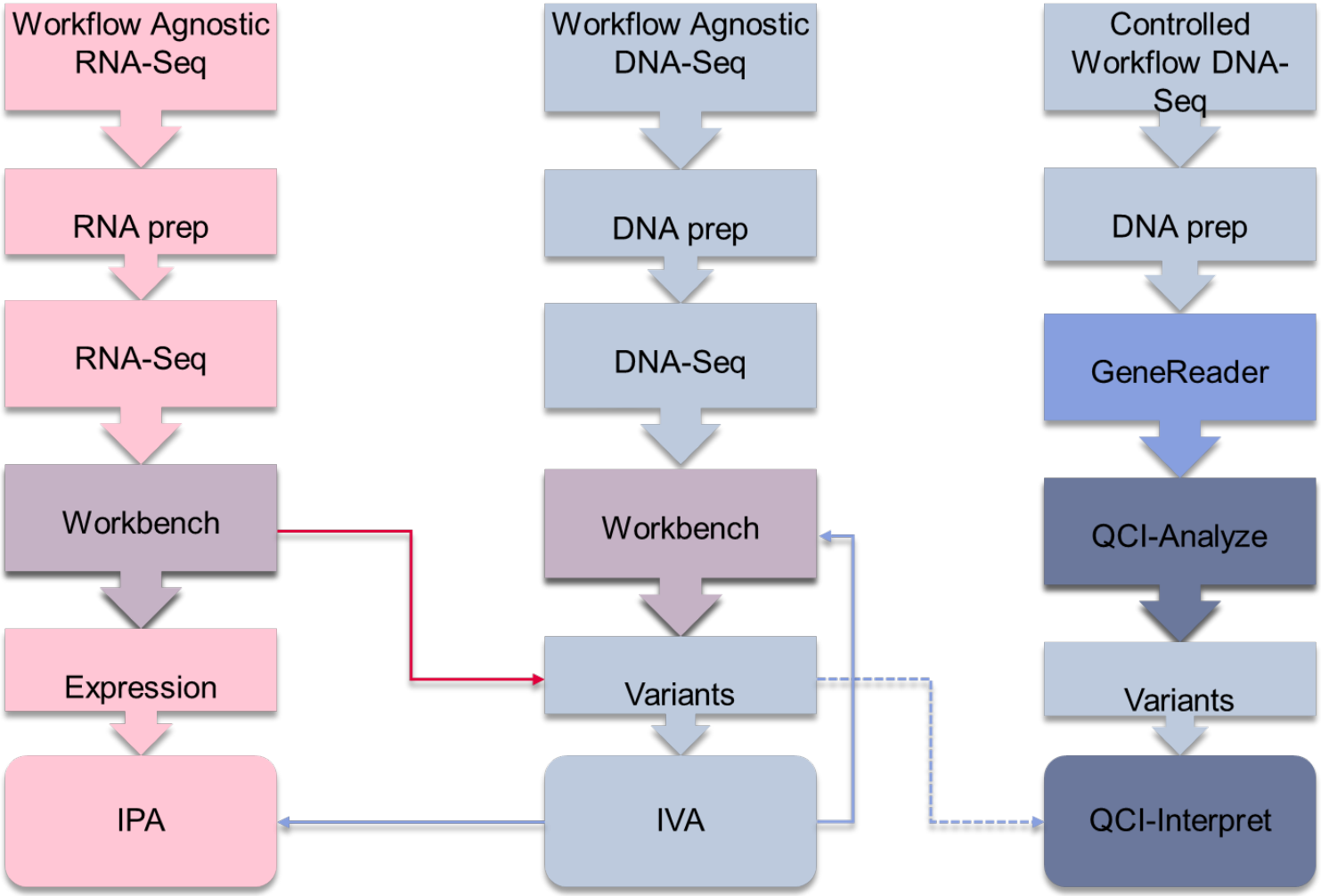
Agenda

- Introduction to the portfolio
- Getting started with IVA
- Filtering a whole exome sample
- QCI I demo
- Working with a trio
- Uploading and sharing samples
- Working with your own data

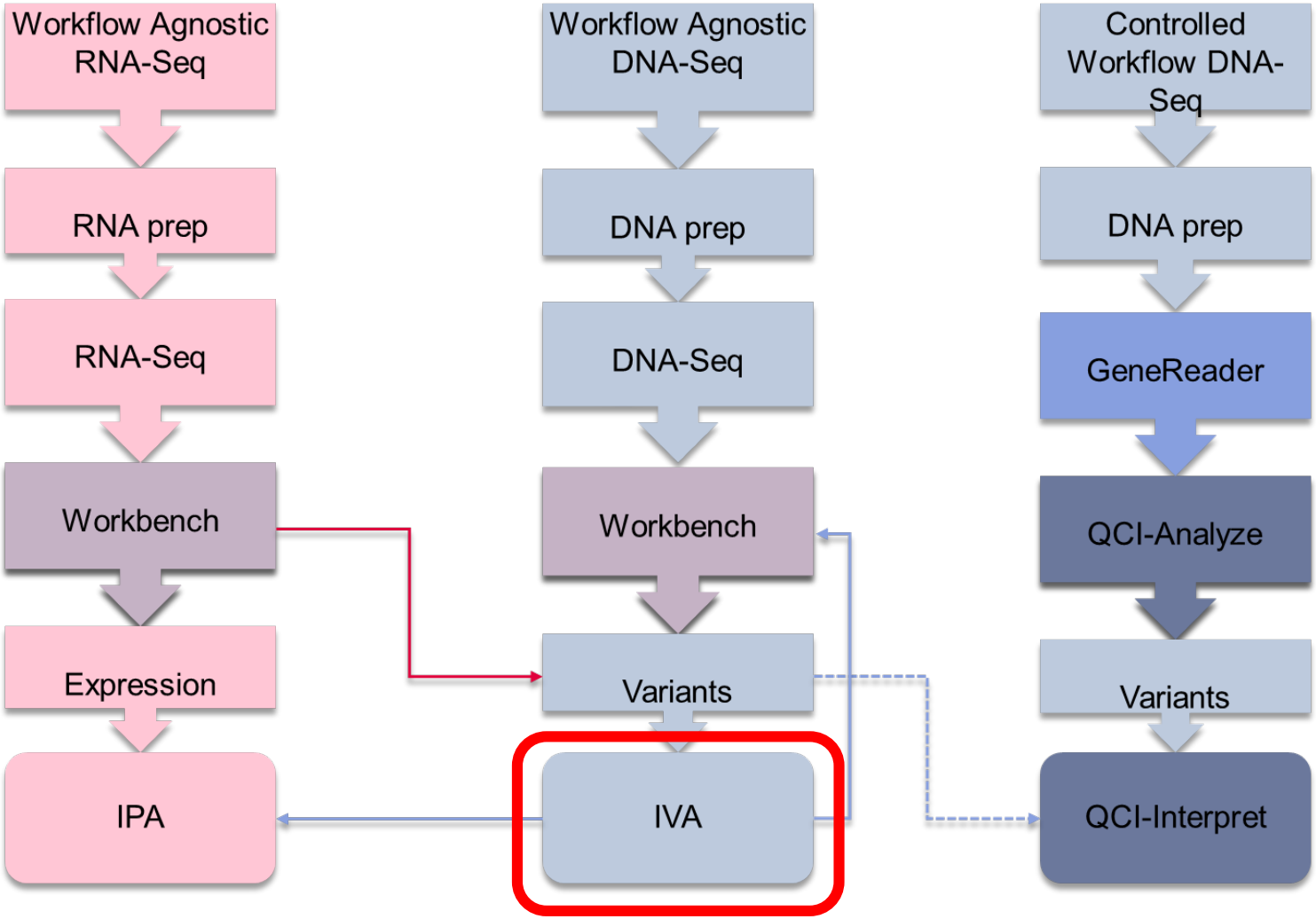
Introduction to the portfolio



Introduction to the portfolio



Introduction to the portfolio



Getting started with IVA

Log-on

https://apps.ingenuity.com

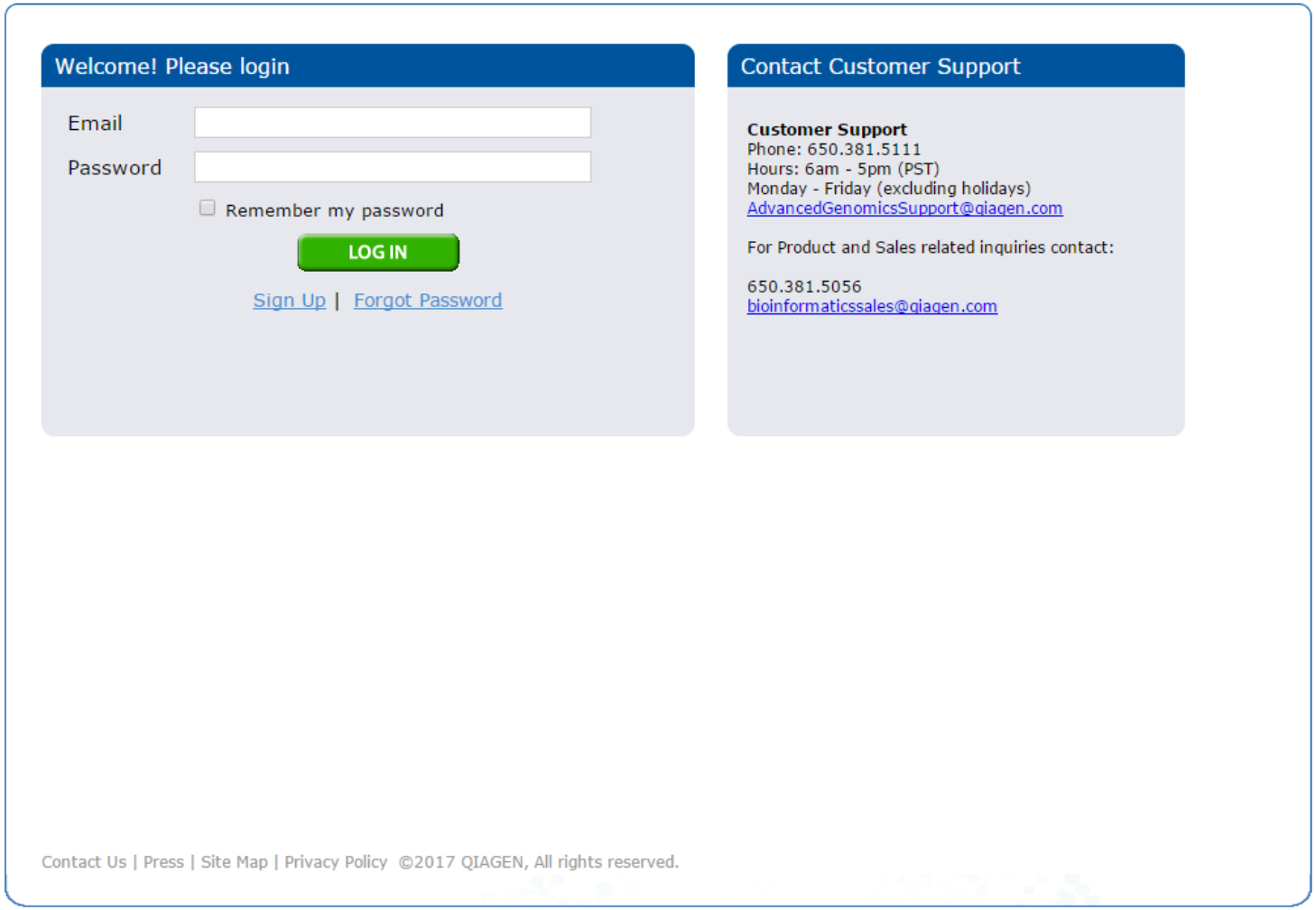
See your sheet for number

Login name:

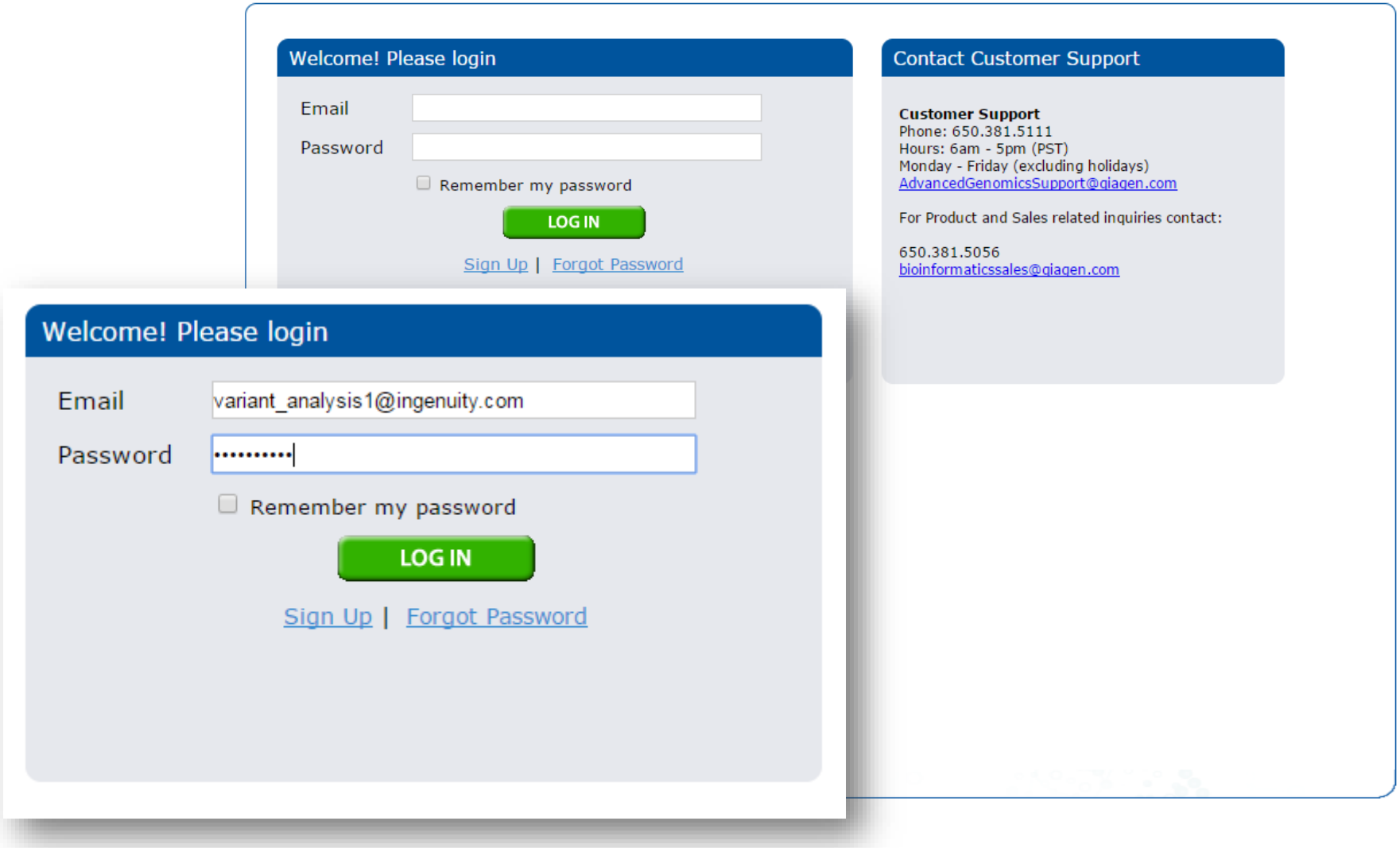
variant_analysis1@ingenuity.com or vatraining1@ingenuity.com

Password: variant123

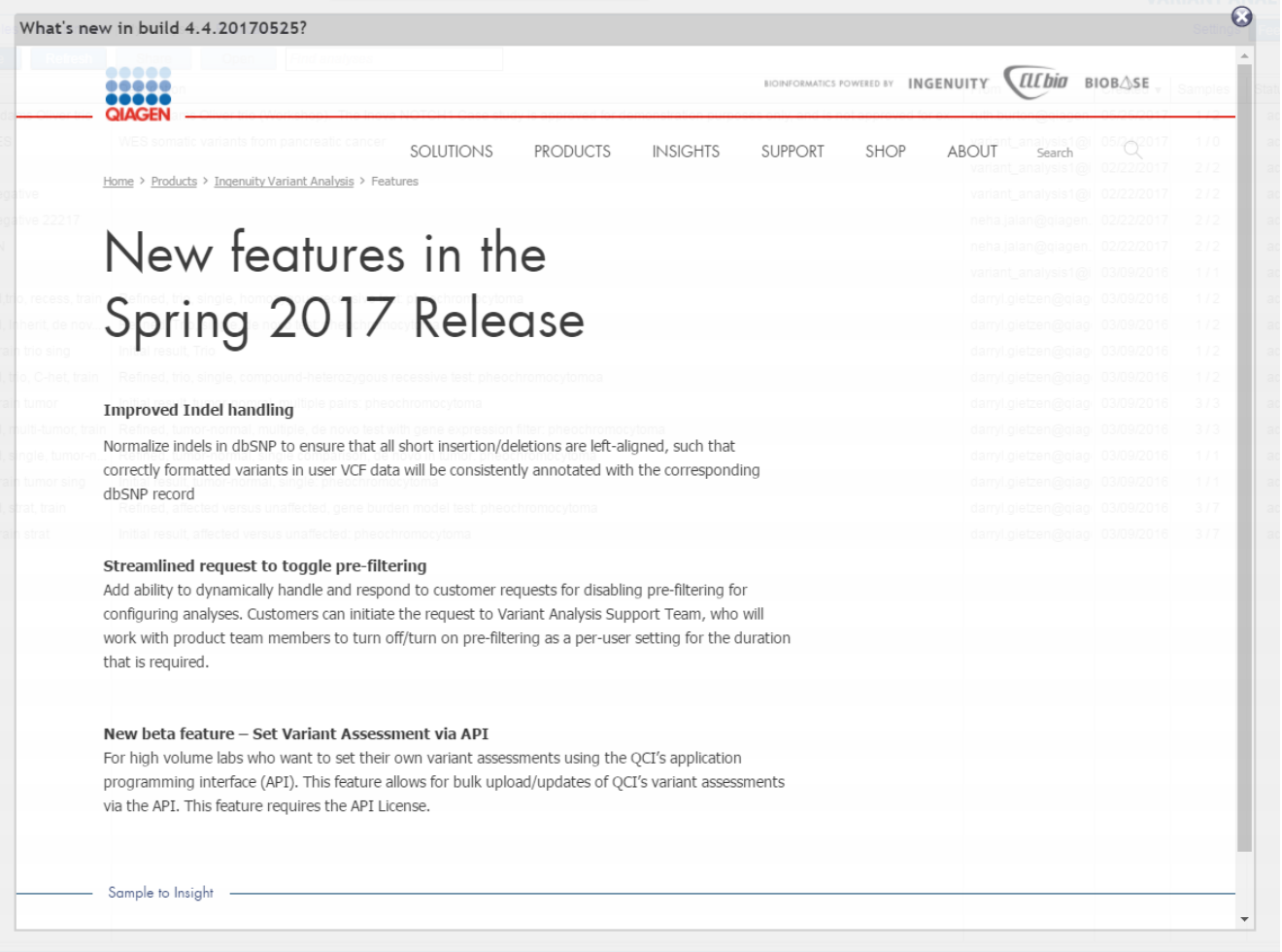
Logging on


Welcome! Please login
Email
Password
Remember my password
LOG IN
Sign Up | Forgot Password
Contact Customer Support
Customer Support
Phone: 650.381.5111
Hours: 6am - 5pm (PST)
Monday - Friday (excluding holidays)
AdvancedGenomicsSupport@qiagen.com
For Product and Sales related inquiries contact:
650.381.5056
bioinformaticssales@qiagen.com
Contact Us | Press | Site Map | Privacy Policy ©2017 QIAGEN, All rights reserved.

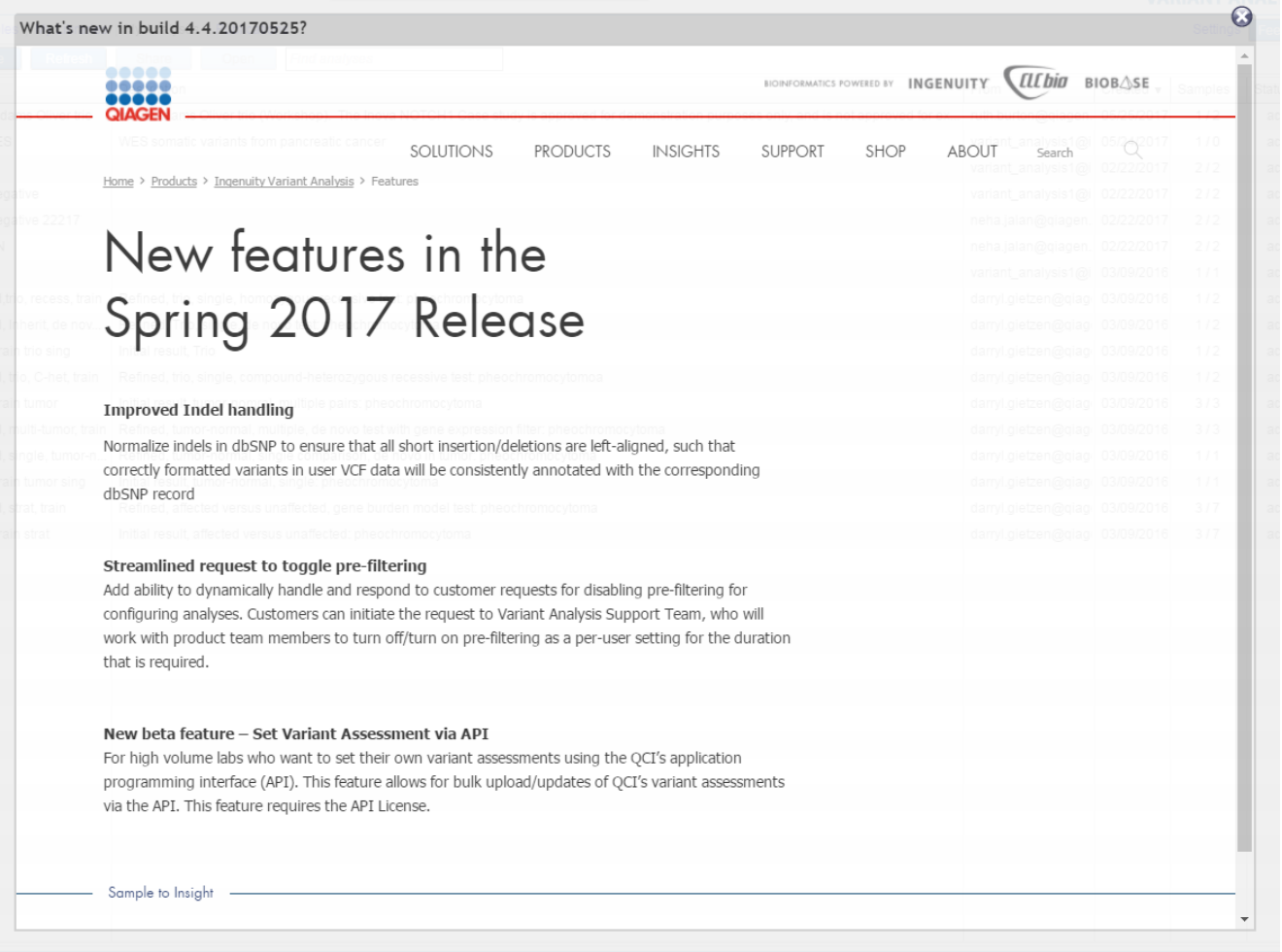
Logging on


Welcome! Please login
Email
Password
Remember my password
LOG IN
Sign Up | Forgot Password
Contact Customer Support
Customer Support
Phone: 650.381.5111
Hours: 6am - 5pm (PST)
Monday - Friday (excluding holidays)
AdvancedGenomicsSupport@qiagen.com
For Product and Sales related inquiries contact:
650.381.5056
bioinformaticssales@qiagen.com
Welcome! Please login
Email
variant_analysis1@ingenuity.com
Password
Remember my password
LOG IN
Sign Up | Forgot Password

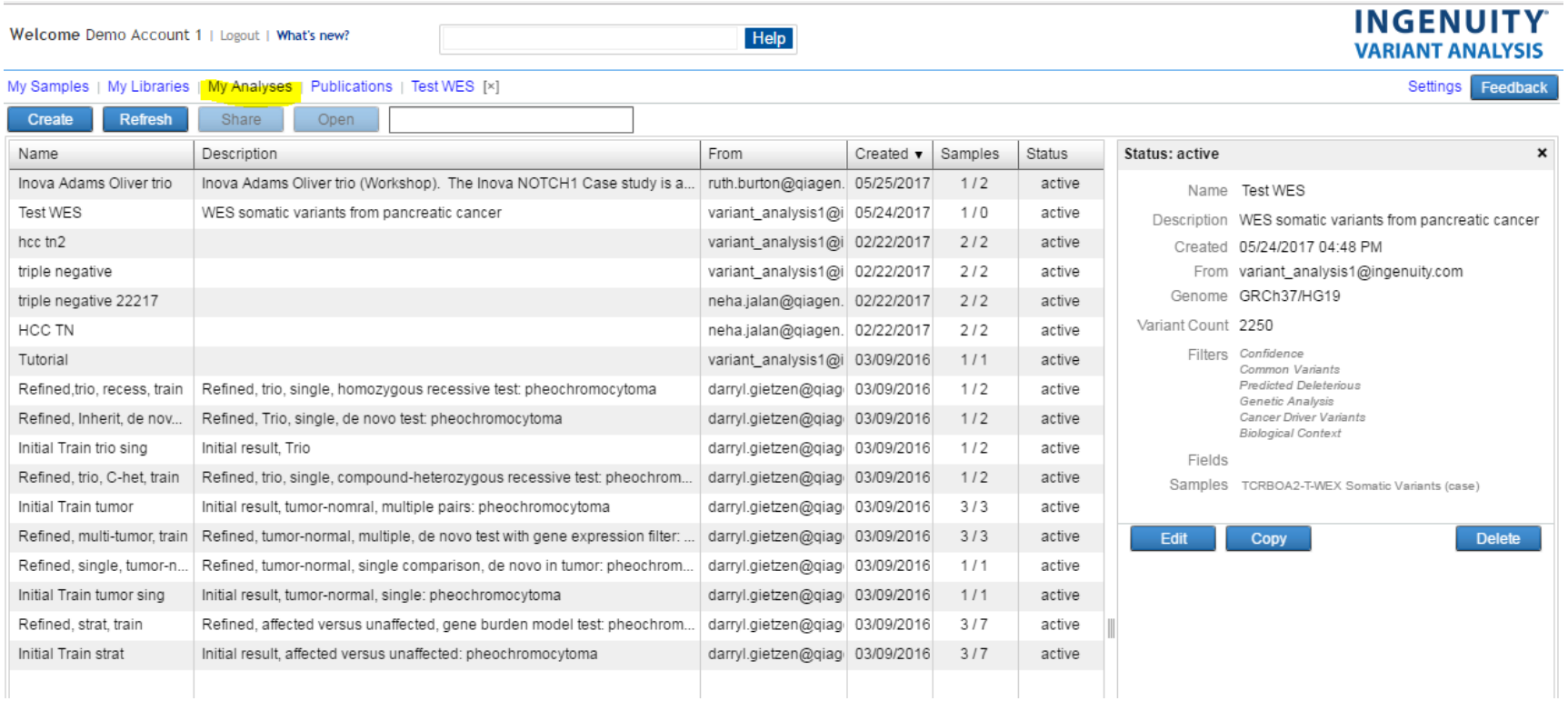
Welcome screen



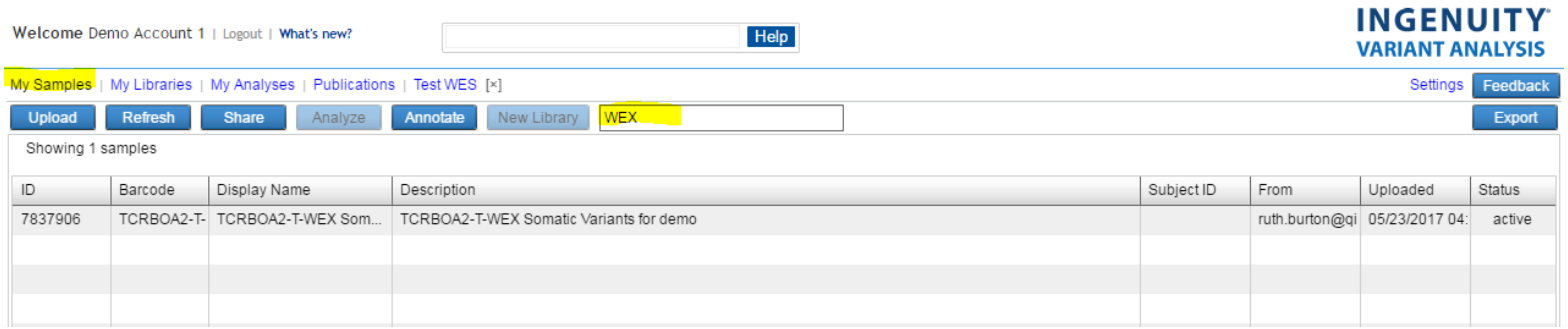
Welcome screen, may see other info such as T&Cs or a sample is waiting to be imported…



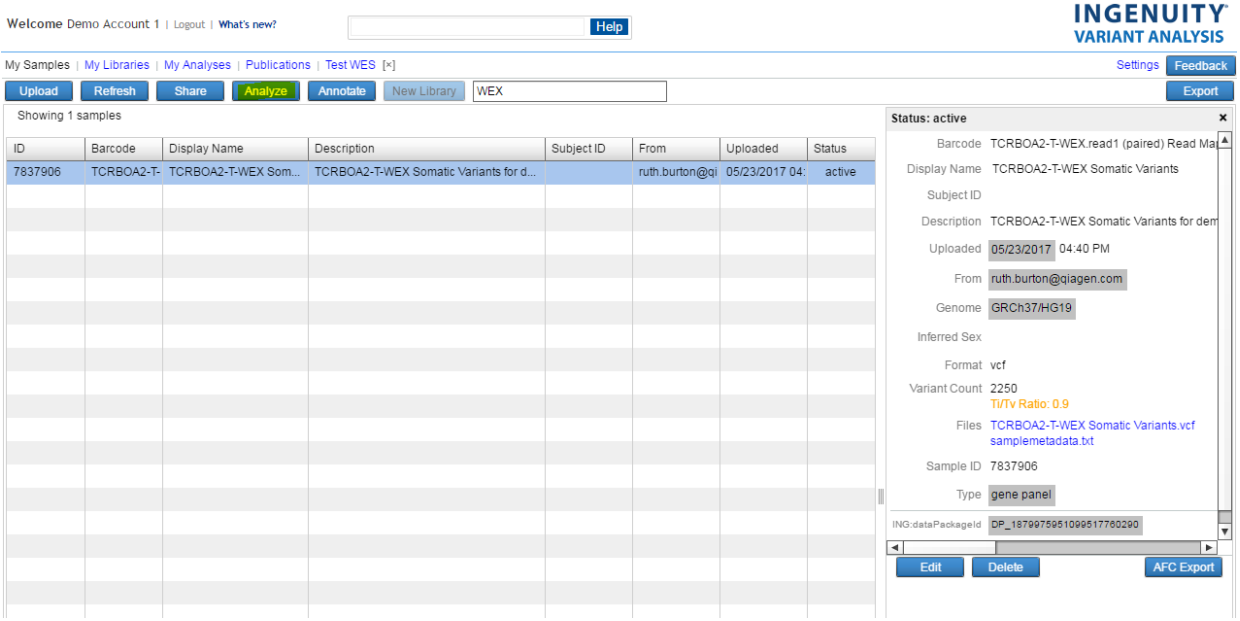
Find the demo sample, the default view is analysis – we need to find the sample



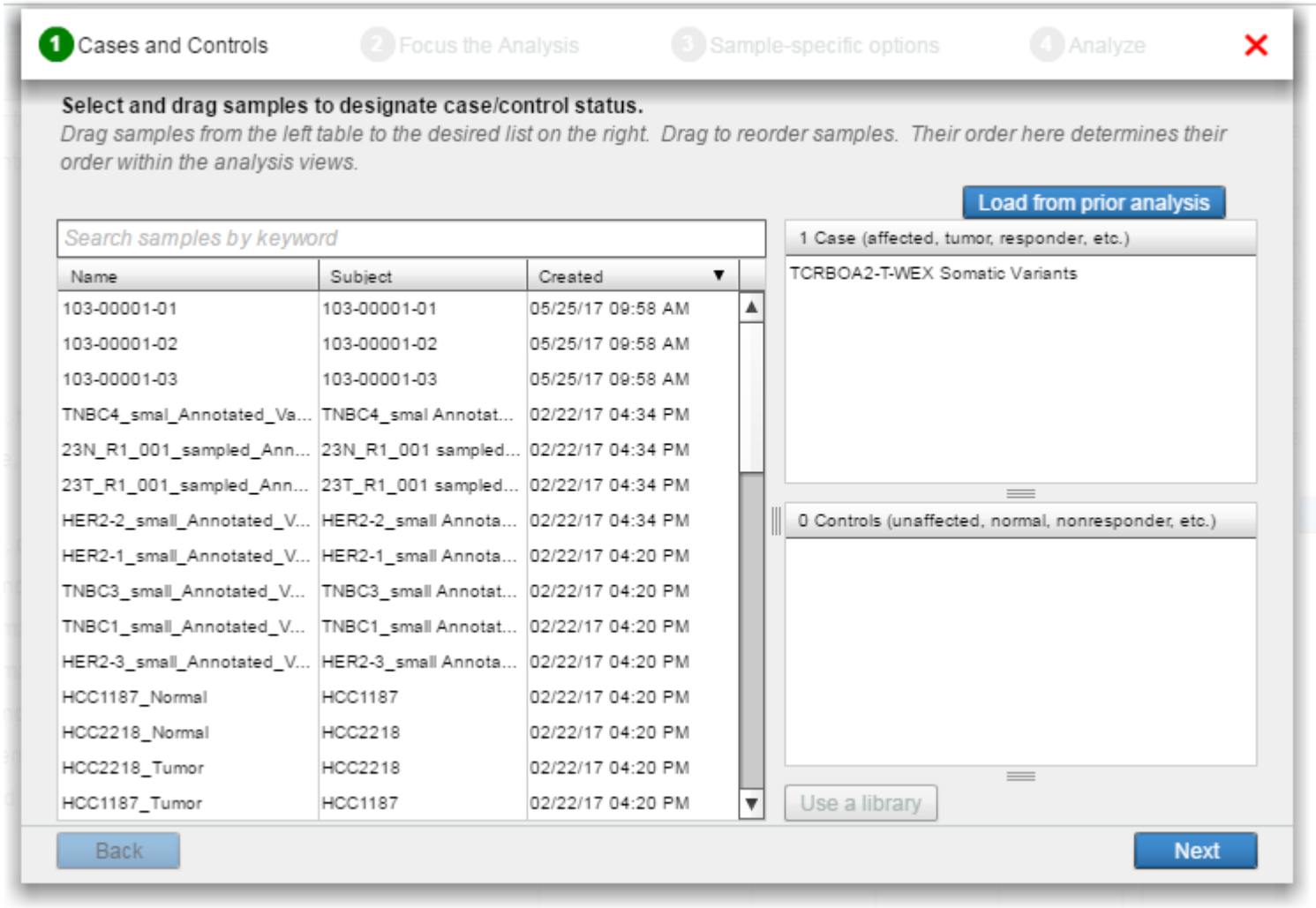
Change to samples and search for “WEX”



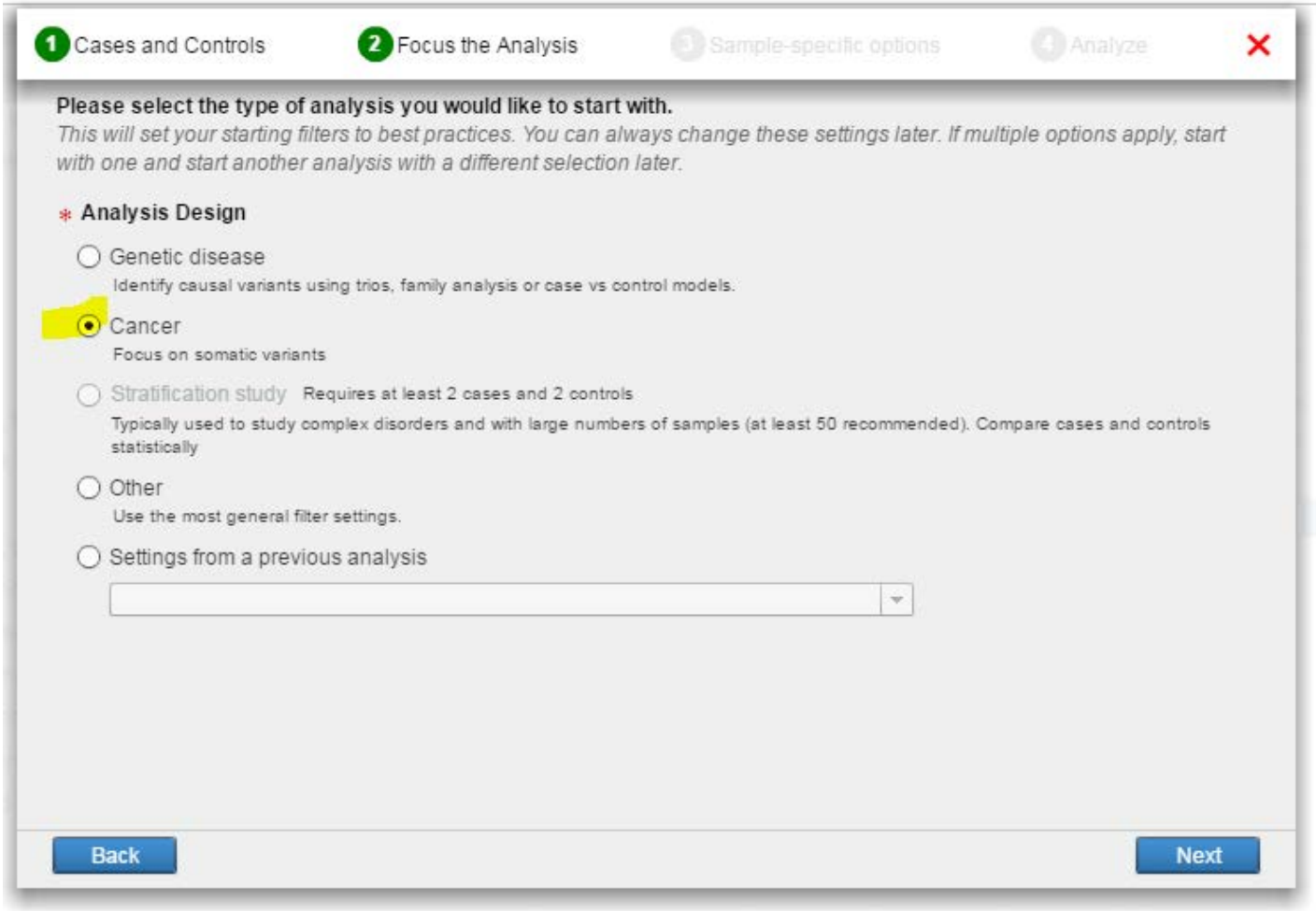
Highlight sample and click “Analyze”



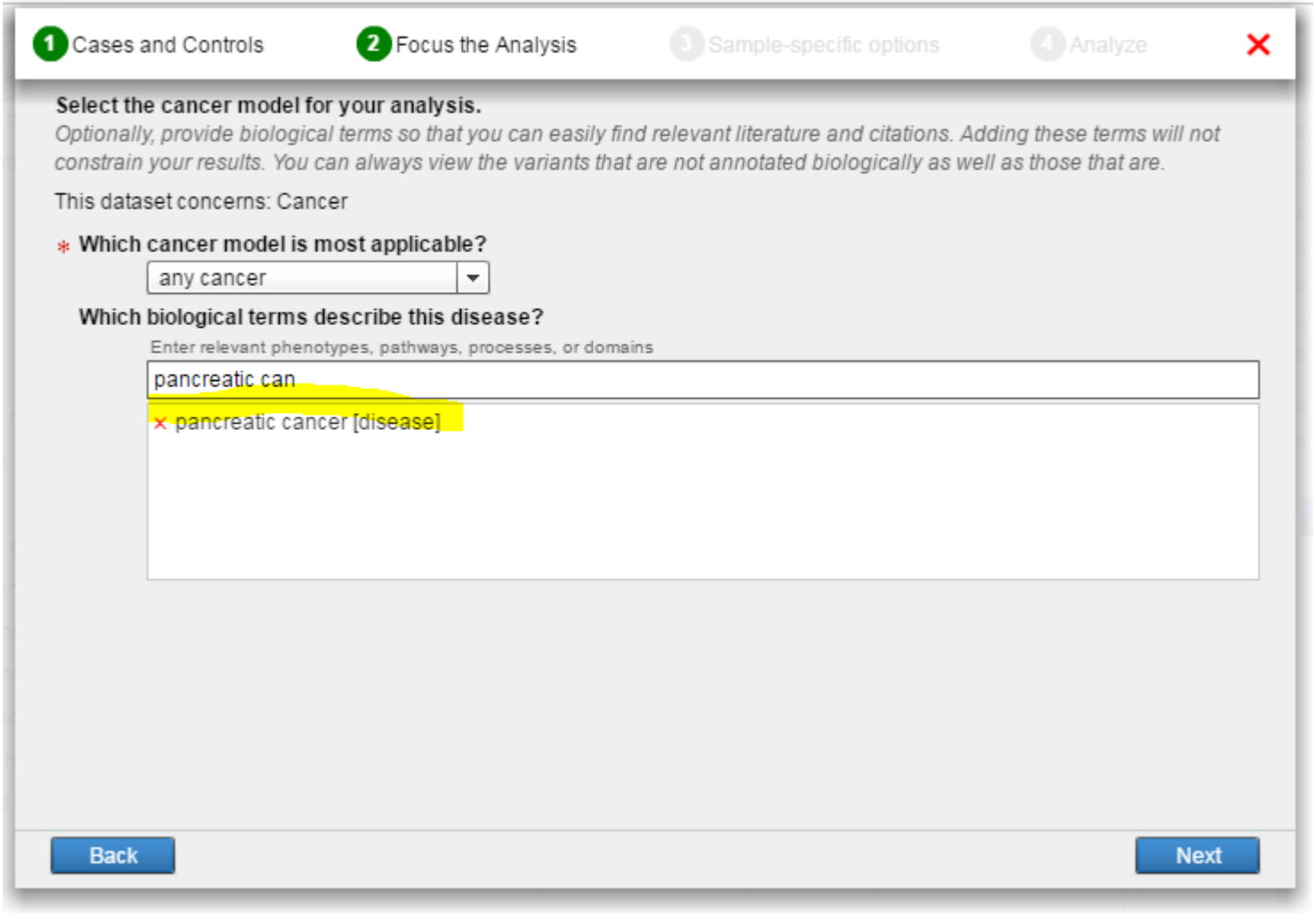
A series of options follows, guiding you through the analysis



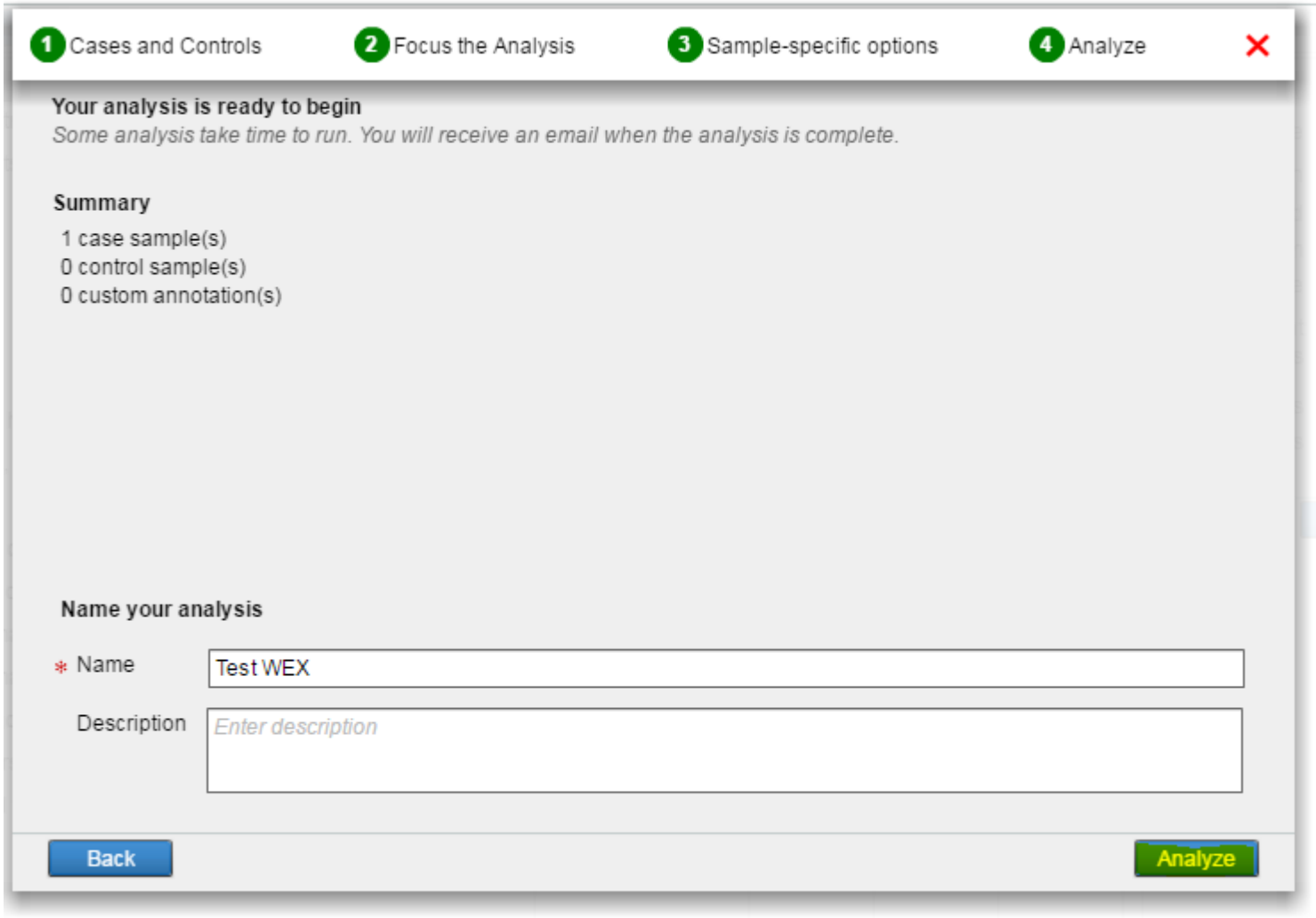
A series of options follows, guiding you through the analysis



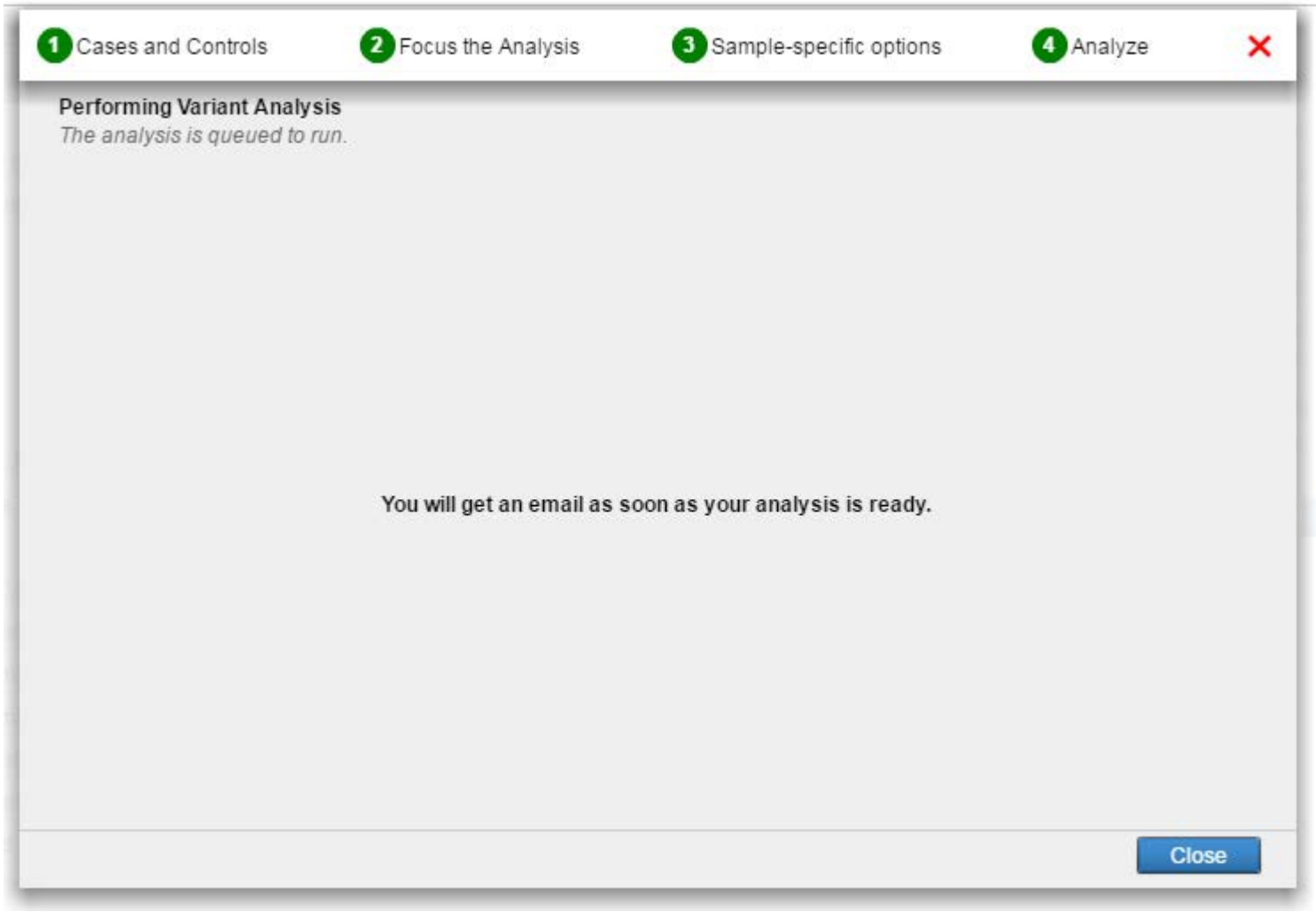
A series of options follows, guiding you through the analysis



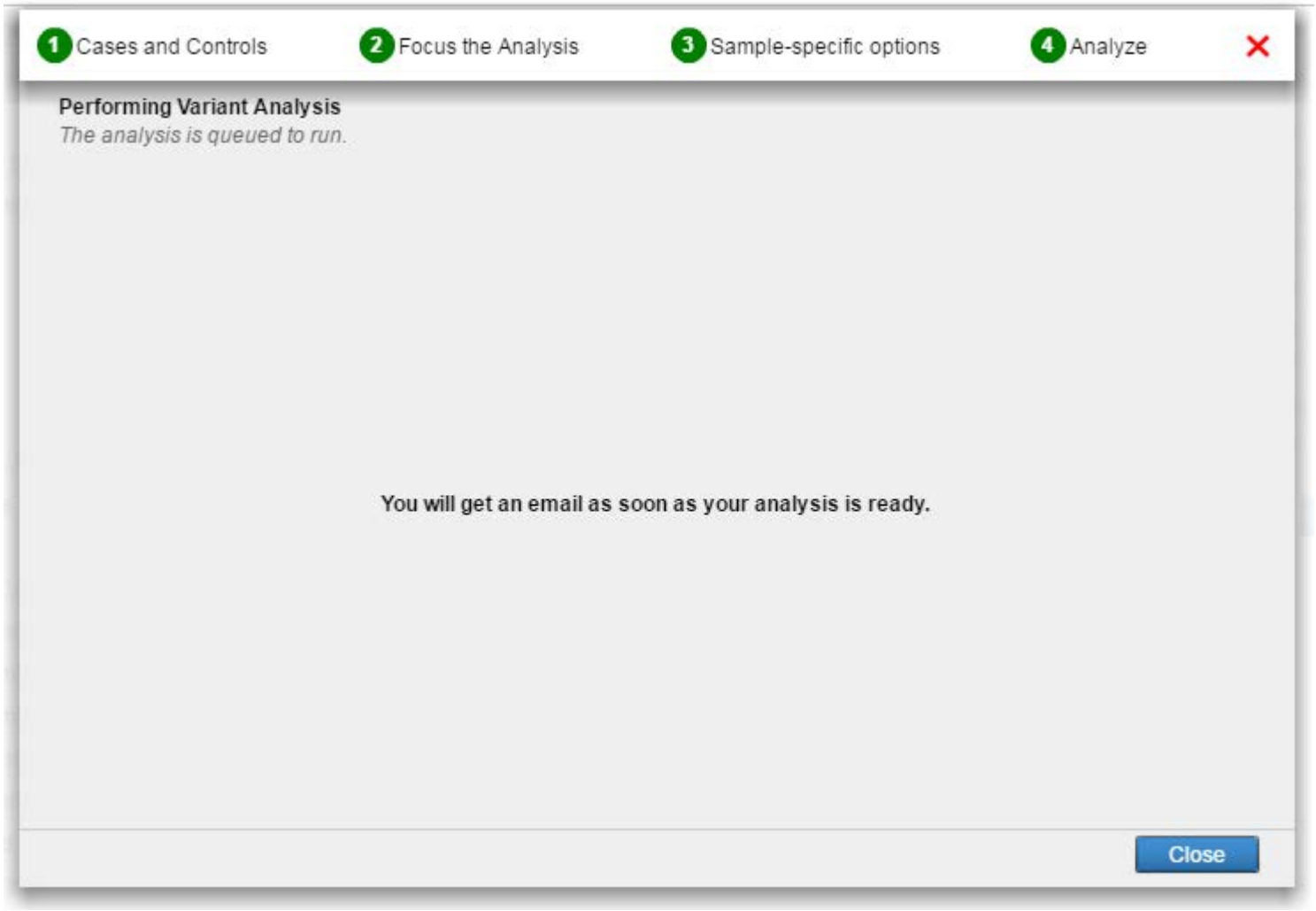
A series of options follows, guiding you through the analysis



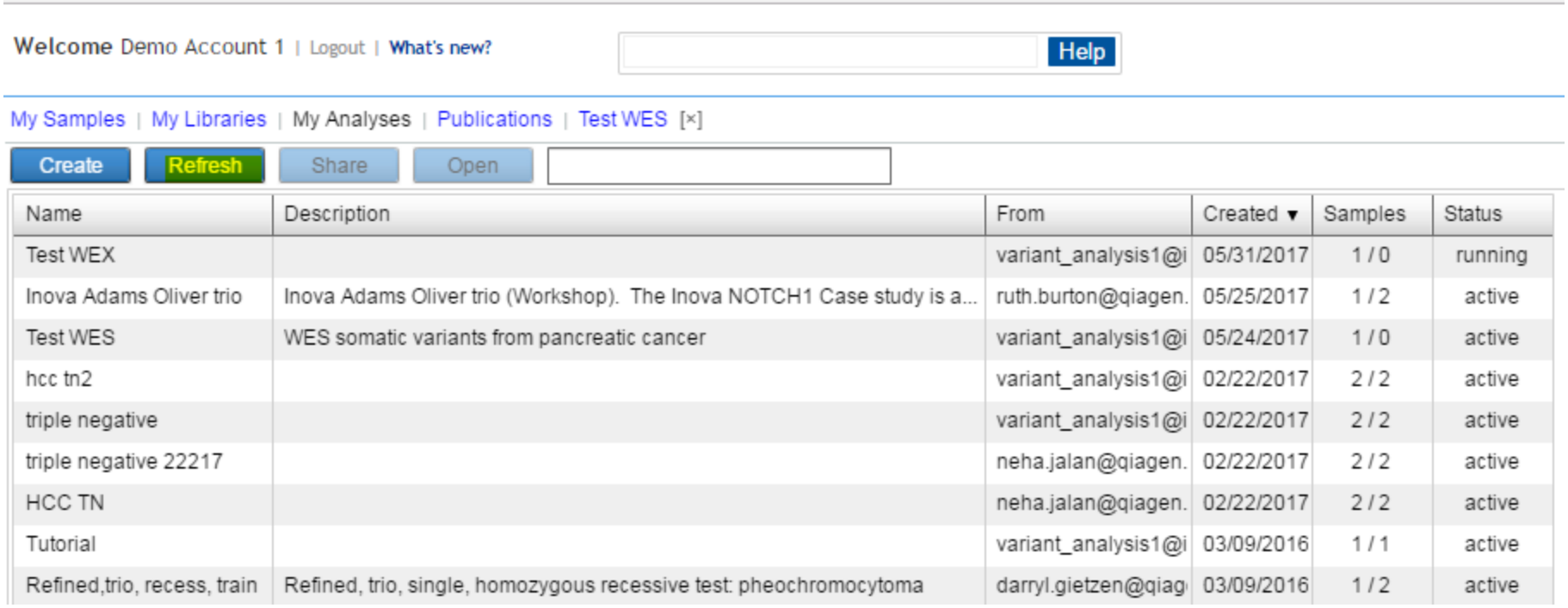
A series of options follows, guiding you through the analysis



A series of options follows, guiding you through the analysis

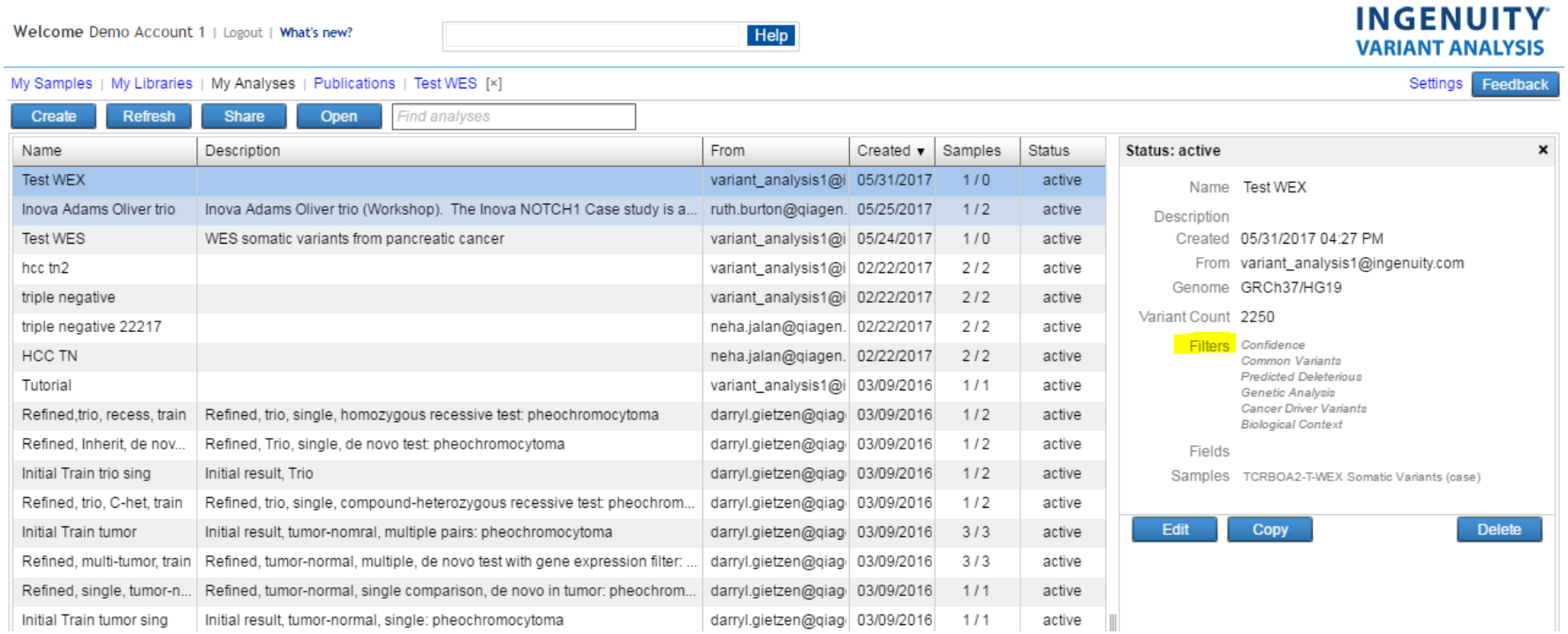


Can wait for the email or press “Refresh”

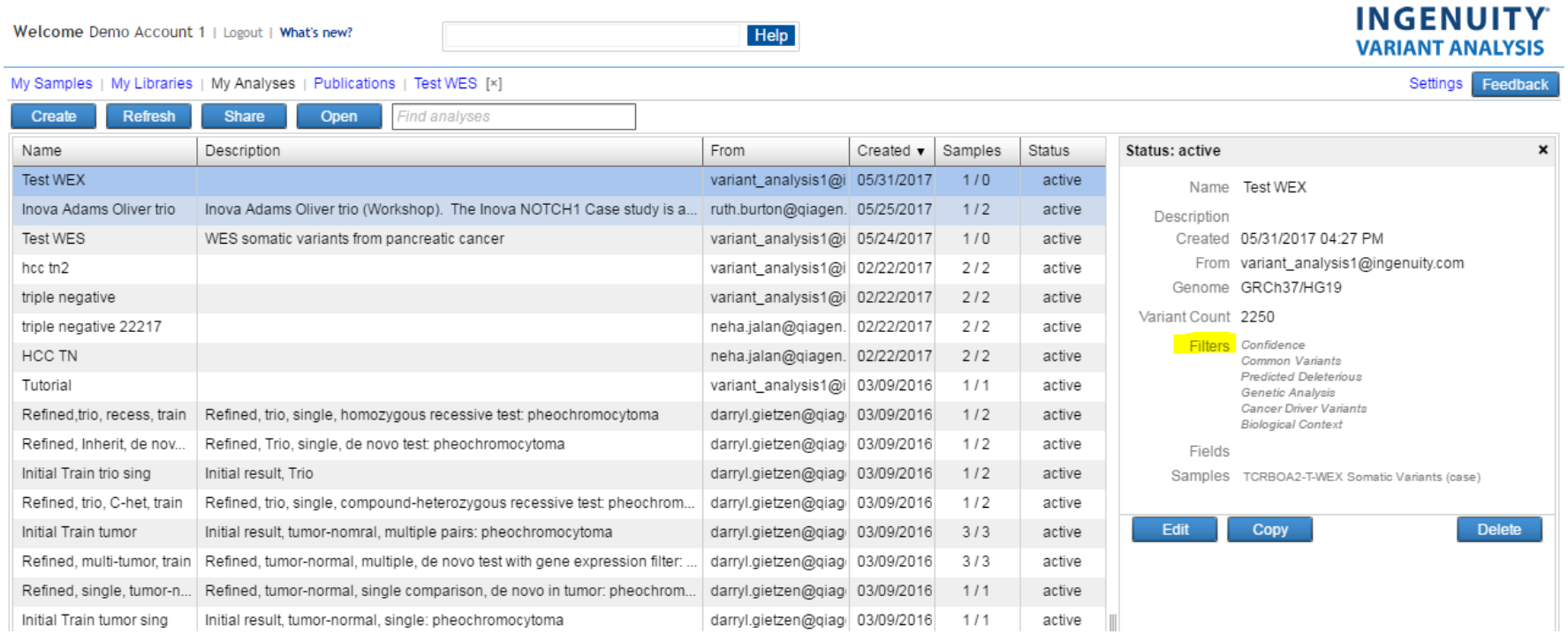


Name	Description	From	Created ▾	Samples	Status
Test WEX		variant_analysis1@i	05/31/2017	1 / 0	running
Inova Adams Oliver trio	Inova Adams Oliver trio (Workshop). The Inova NOTCH1 Case study is a...	ruth.burton@qiagen.	05/25/2017	1 / 2	active
Test WES	WES somatic variants from pancreatic cancer	variant_analysis1@i	05/24/2017	1 / 0	active
hcc tn2		variant_analysis1@i	02/22/2017	2 / 2	active
triple negative		variant_analysis1@i	02/22/2017	2 / 2	active
triple negative 22217		neha.jalan@qiagen.	02/22/2017	2 / 2	active
HCC TN		neha.jalan@qiagen.	02/22/2017	2 / 2	active
Tutorial		variant_analysis1@i	03/09/2016	1 / 1	active
Refined,trio, recess, train	Refined, trio, single, homozygous recessive test: pheochromocytoma	darryl.gietzen@qiag	03/09/2016	1 / 2	active

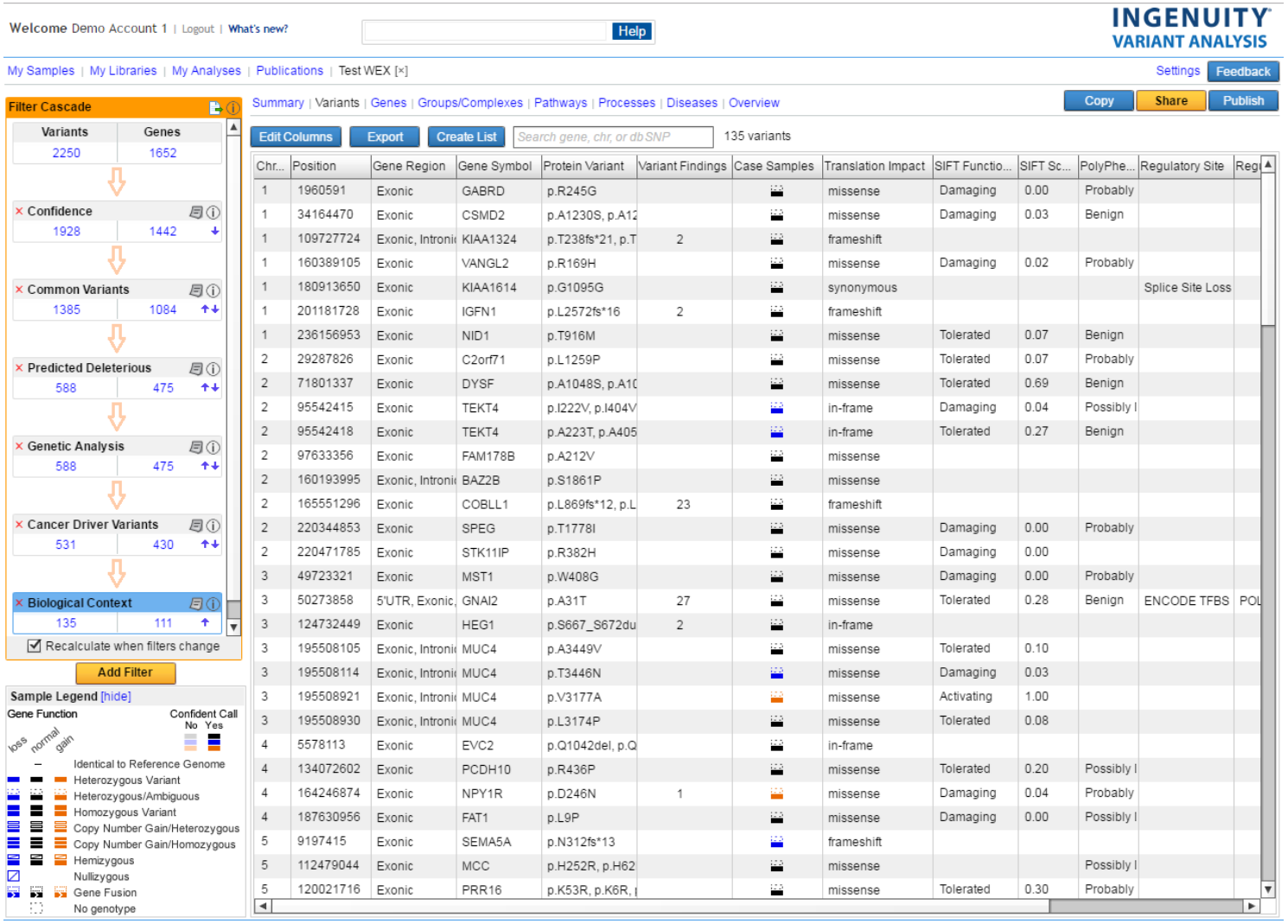
When active can open analysis and review the filtering steps



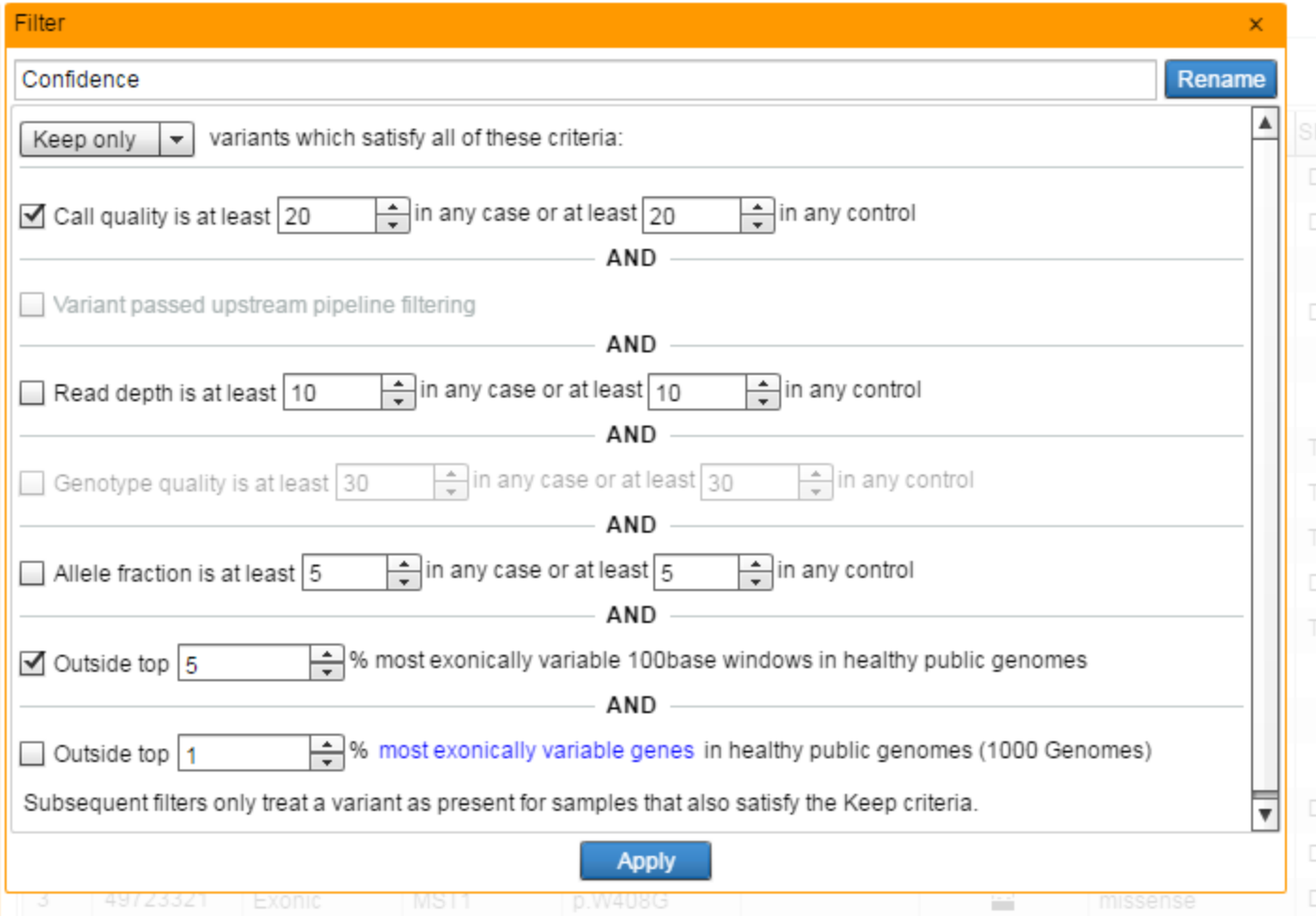
When active can open analysis and review the filtering steps



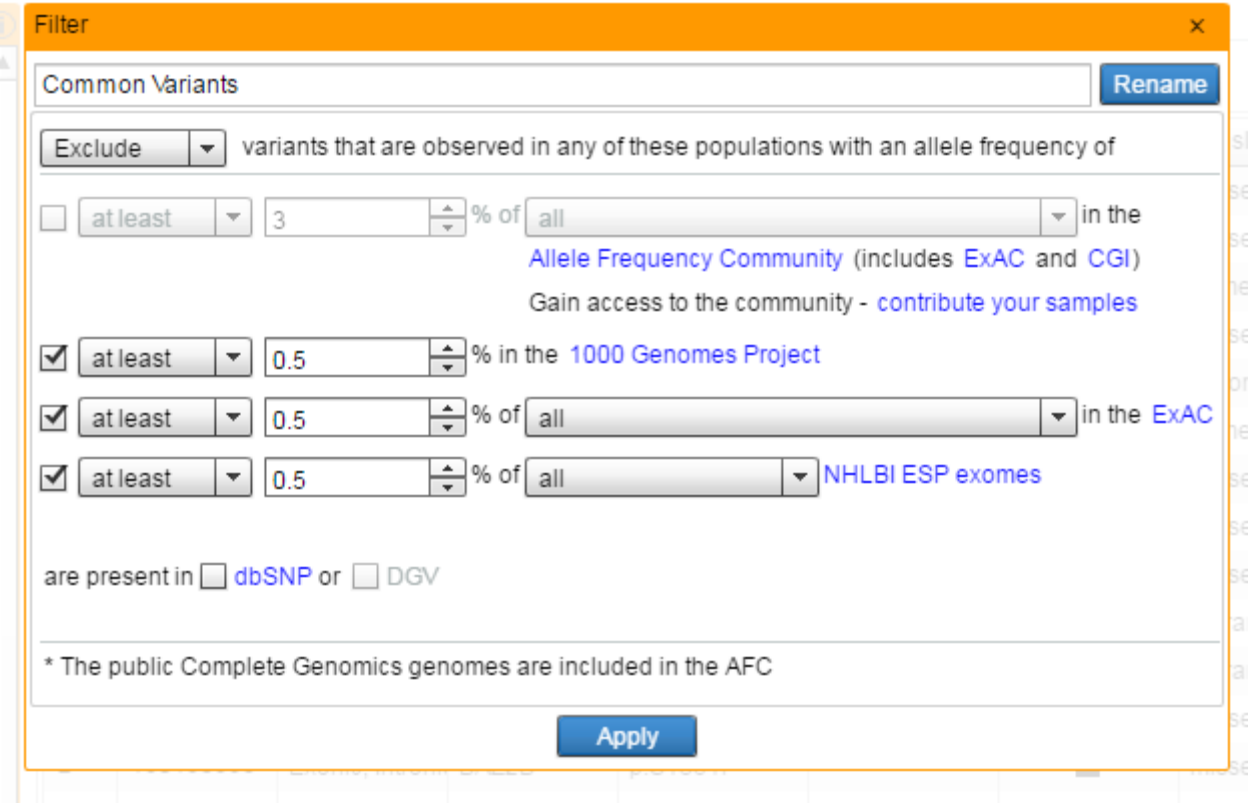
A filtering cascade has been applied… let's look at the filters in order



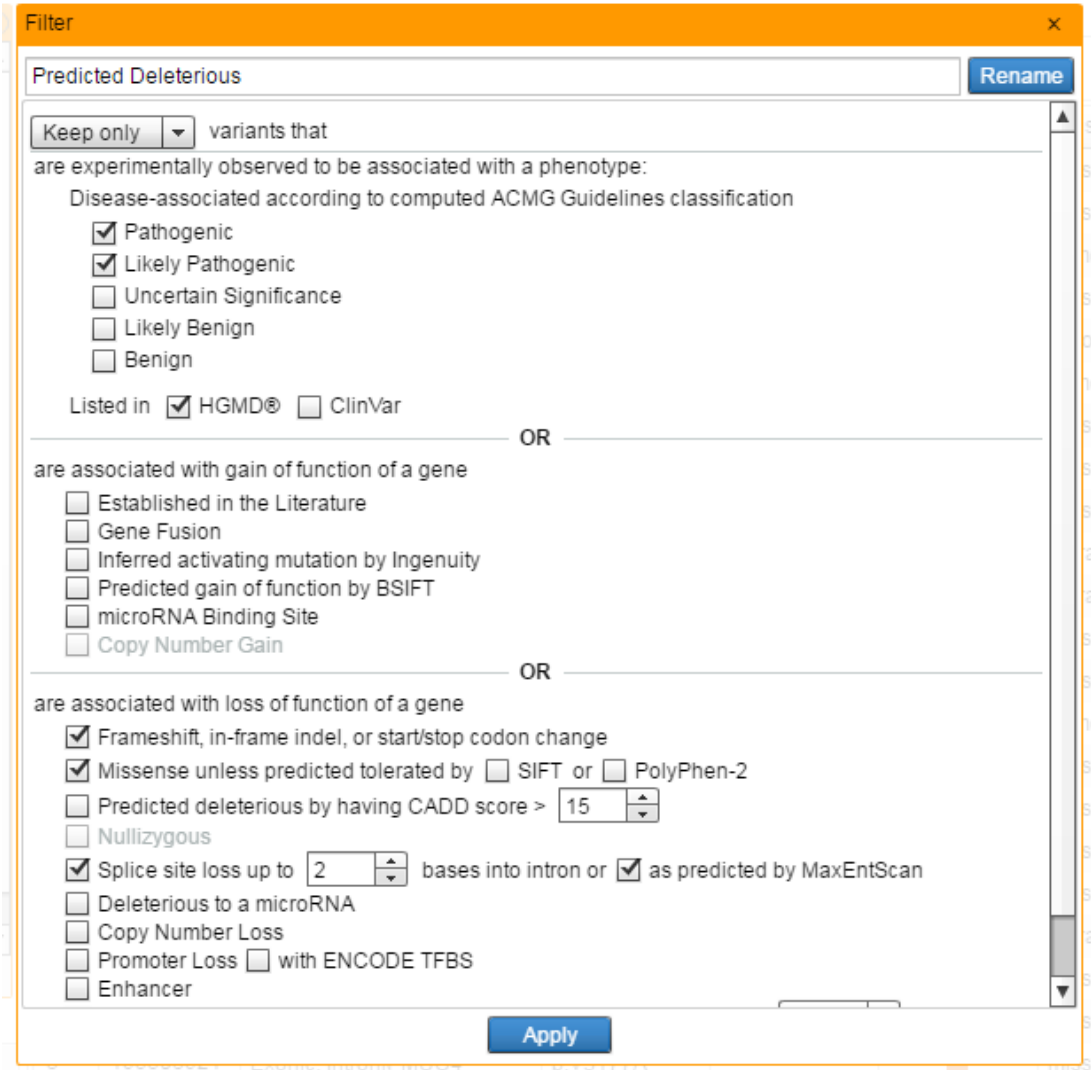
A filtering cascade has been applied… let's look at the filters in order



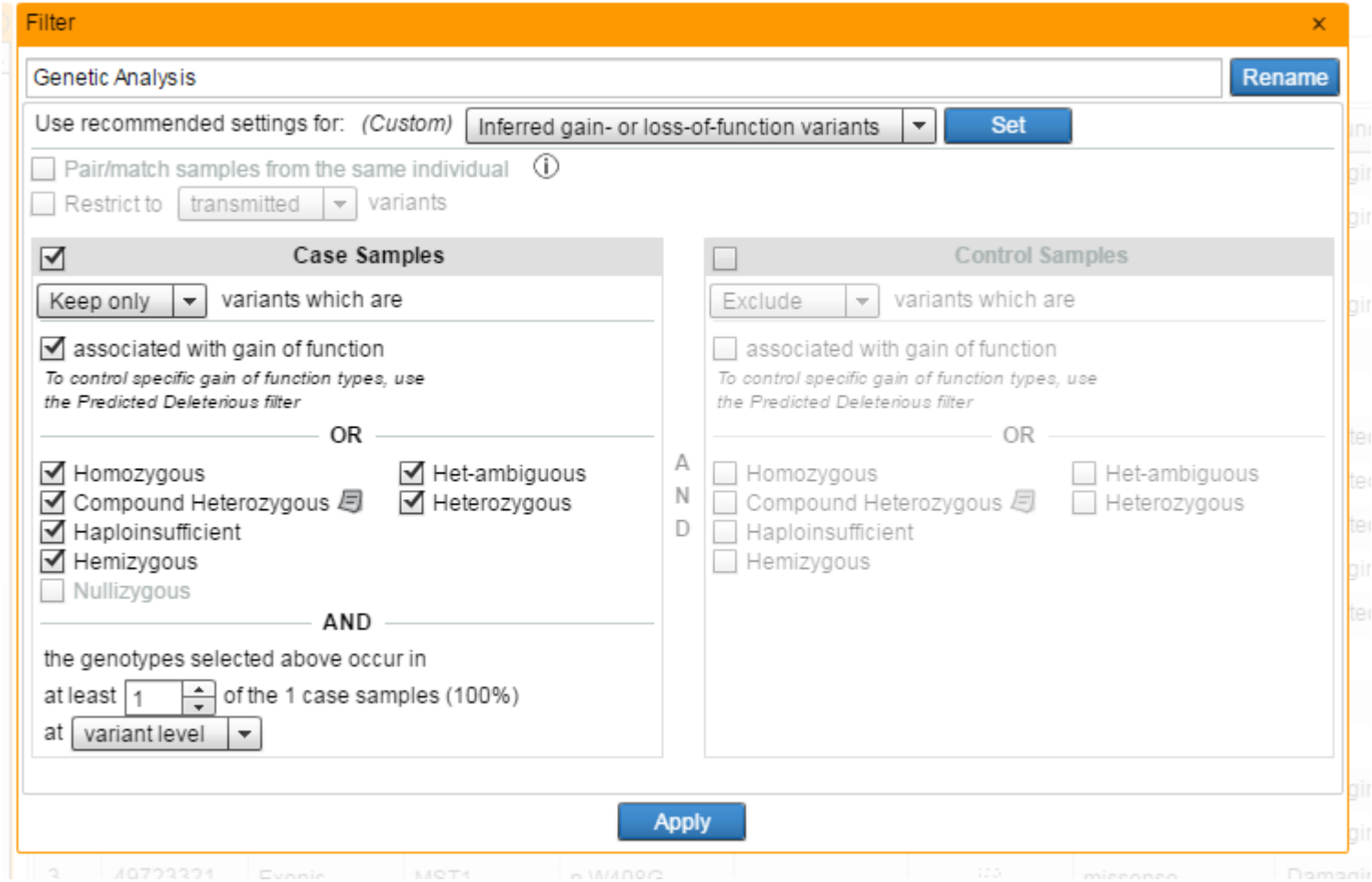
A filtering cascade has been applied… let's look at the filters in order



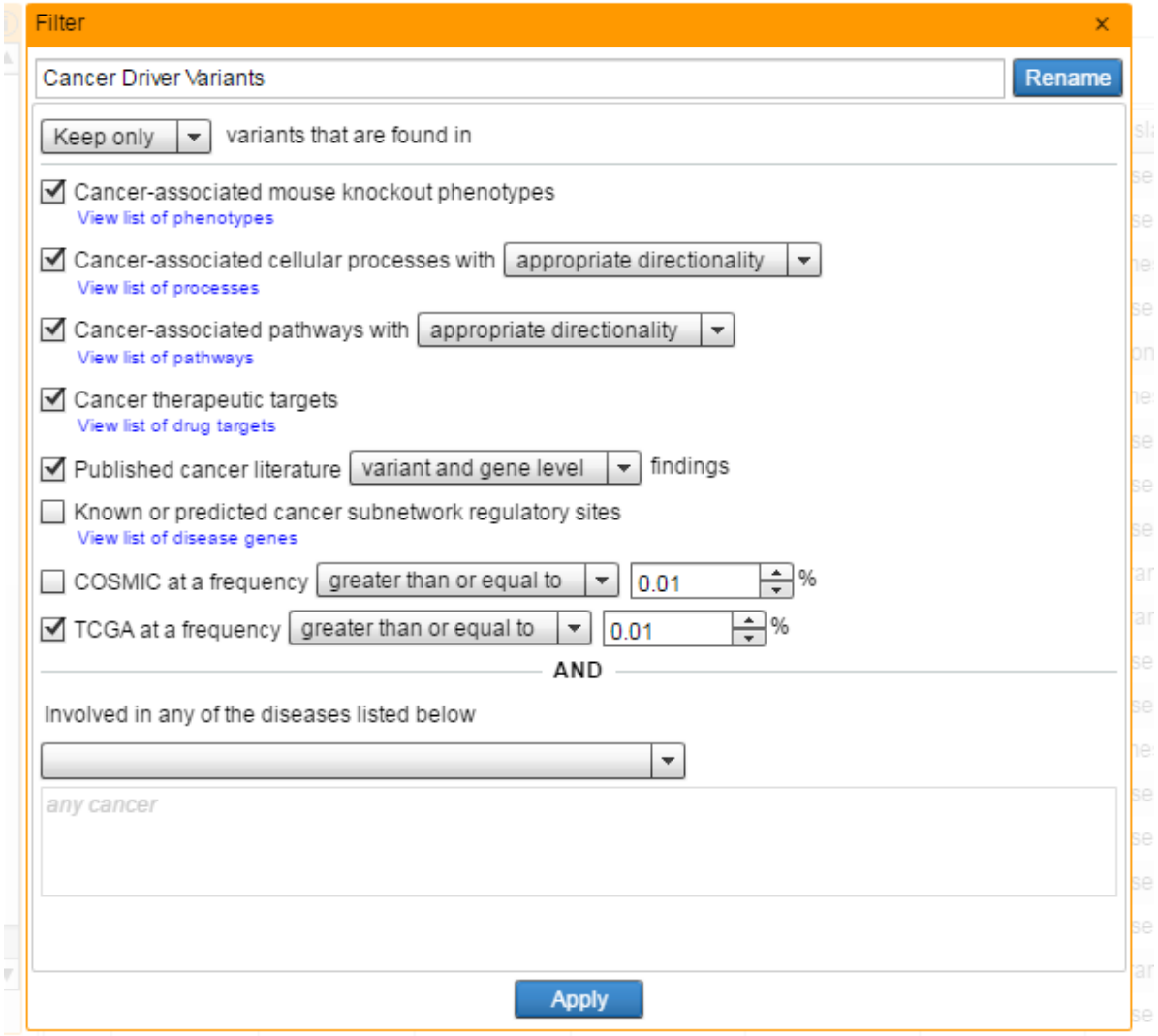
A filtering cascade has been applied… let's look at the filters in order



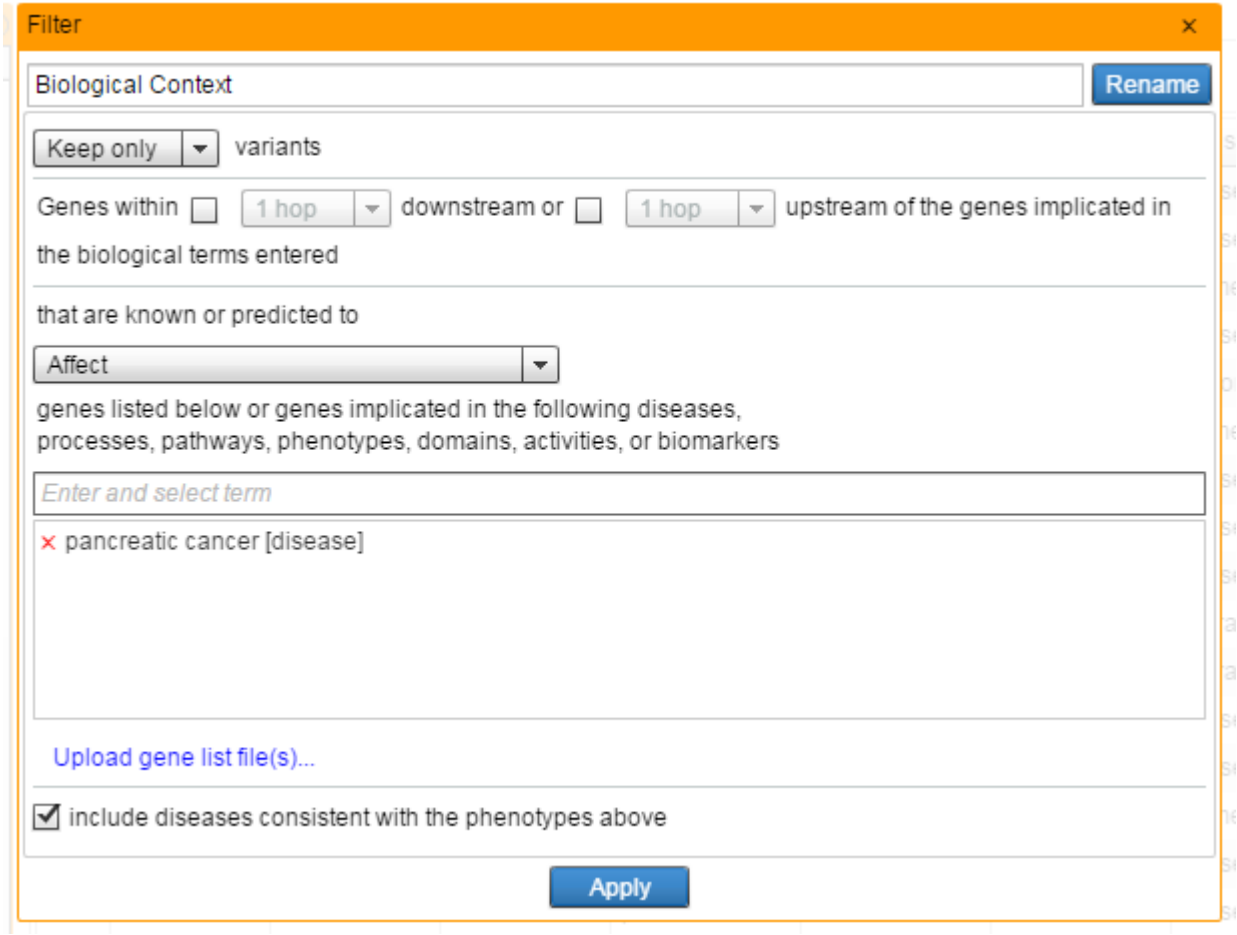
A filtering cascade has been applied… let's look at the filters in order



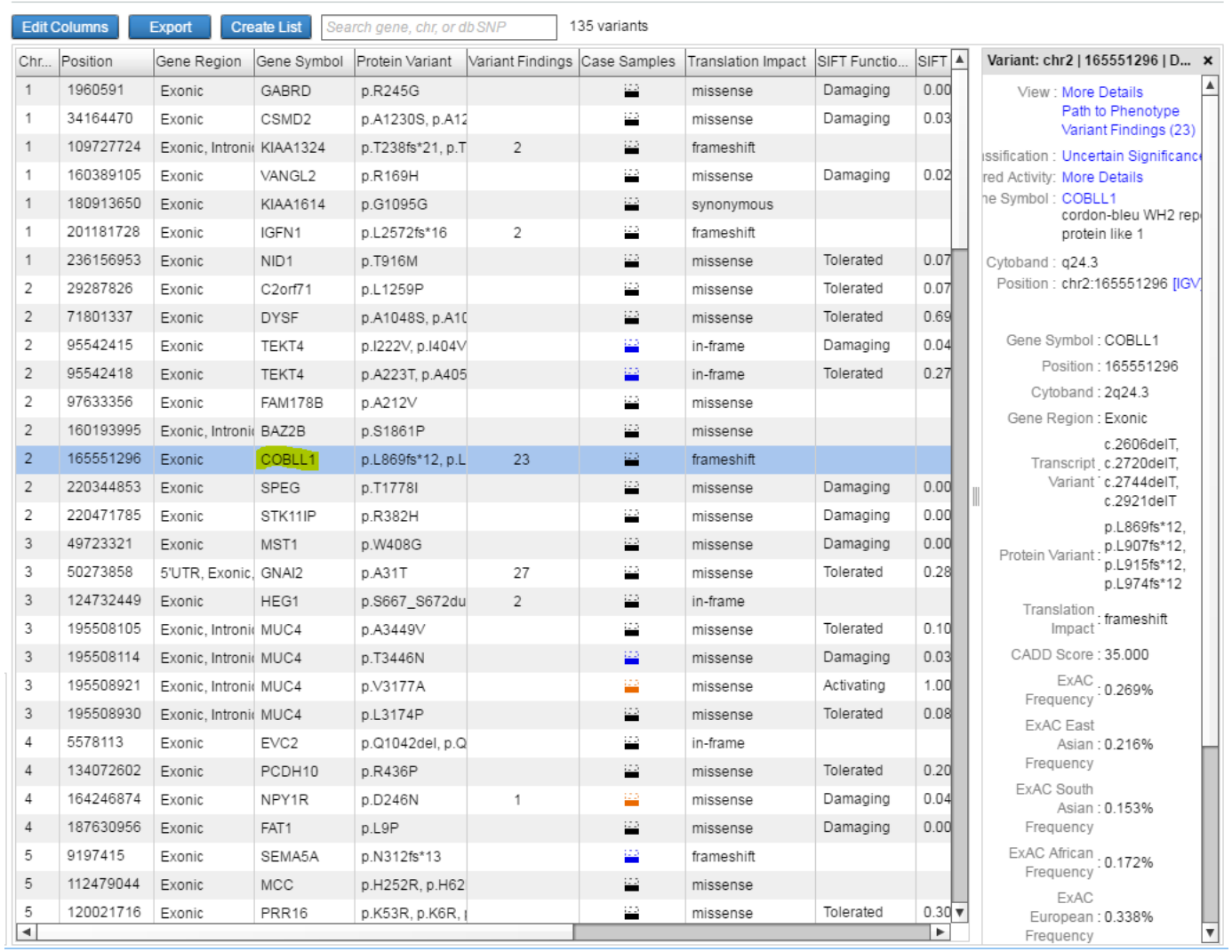
A filtering cascade has been applied… let's look at the filters in order



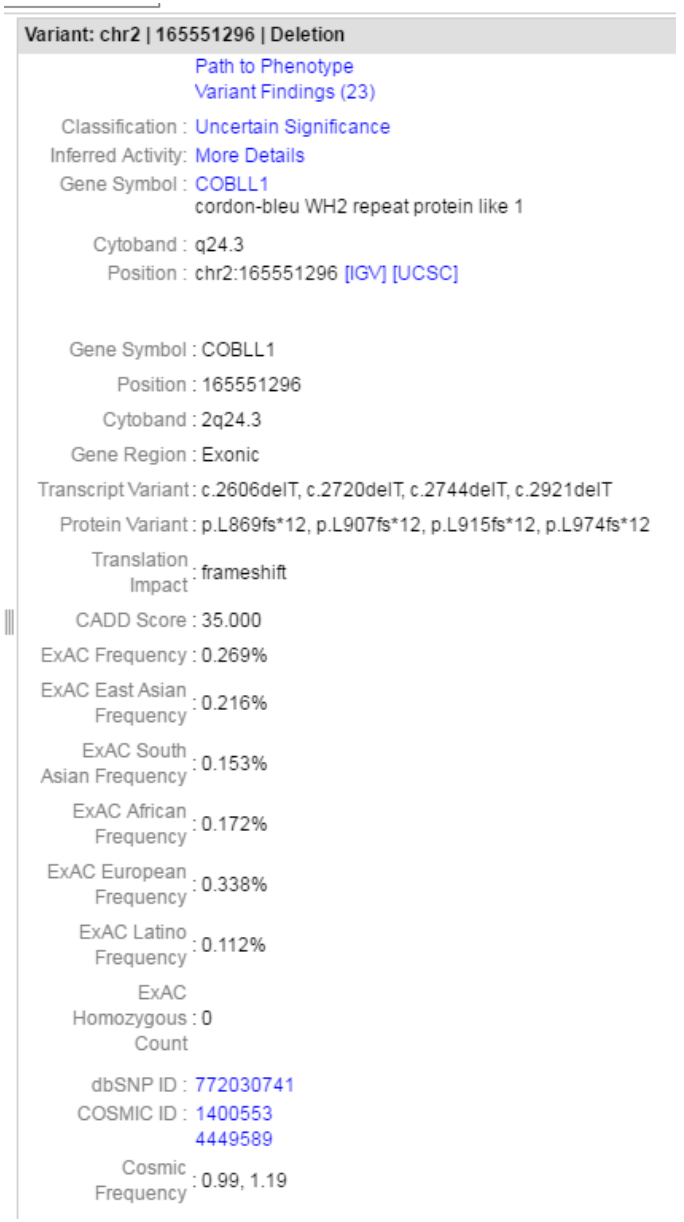
A filtering cascade has been applied… let's look at the filters in order



As well as filtering IVA provides a wealth of information…



As well as filtering IVA provides a wealth of information…



Variant: chr2 | 165551296 | Deletion

Path to Phenotype
Variant Findings (23)

Classification : Uncertain Significance
Inferred Activity: More Details
Gene Symbol : COBLL1
cordon-bleu WH2 repeat protein like 1

Cytoband : q24.3
Position : chr2:165551296 [IGV] [UCSC]

Gene Symbol	COBLL1
Position	165551296
Cytoband	2q24.3
Gene Region	Exonic
Transcript Variant	c.2606delT, c.2720delT, c.2744delT, c.2921delT
Protein Variant	p.L869fs*12, p.L907fs*12, p.L915fs*12, p.L974fs*12
Translation Impact	frameshift
CADD Score	35.000
ExAC Frequency	0.269%
ExAC East Asian Frequency	0.216%
ExAC South Asian Frequency	0.153%
ExAC African Frequency	0.172%
ExAC European Frequency	0.338%
ExAC Latino Frequency	0.112%
ExAC Homozygous Count	0
dbSNP ID	772030741
COSMIC ID	1400553 4449589
Cosmic Frequency	0.99, 1.19

As well as filtering IVA provides a wealth of information…

Variant: chr2 | 165551296 | Deletion (COBLL1 p.L869fs*12, etc) - Computed Classification Explana...

Computed classification based on ACMG guidelines: **Uncertain Significance for Schizophrenia**

Evidence for pathogenicity

- PP3 - Multiple lines of computational evidence support a deleterious effect on the gene or gene product [CADD = 35.0] (Supporting)

Evidence against pathogenicity

- none

IPA Gene View: COBLL1

Summary

Entrez Gene Name:	cordon-bleu WH2 repeat protein like 1
Synonym(s):	1810047P18RIK, Cobl-like 1, COBLR1, cordon-bleu WH2 repeat protein-like 1, D430044D16Rik, KIAA0977
Protein Functions / Functional Domains:	cadherin binding, protein binding
Subcellular Location:	cell-cell adherens junctions, Extracellular Space, plasma, vesicles
Canonical Pathway:	--

Top findings from Ingenuity Knowledge Base

regulates:	--
regulated by:	IL4, GMNN, SOX3, SOX2, MYC, KLF4, POU5F1, SOX1, Mycobacterium tuberculosis H37Rv, MAP3K8, FOS, Sos
binds:	PACSIN3, KIAA0368, PKM, ASMTL, DYNC1LI1, JUP, PPP4R3A, KIAA1107, TGS1, RASSF1, ITGB3BP, DLG3, DYNC2H1, BRINP1, SORT1
role in cell:	--
disease:	non-insulin-dependent diabetes mellitus, gastric epithelial cancer, gastric carcinoma, colorectal carcinoma

Human Isoforms From RefSeq More Info

Loading

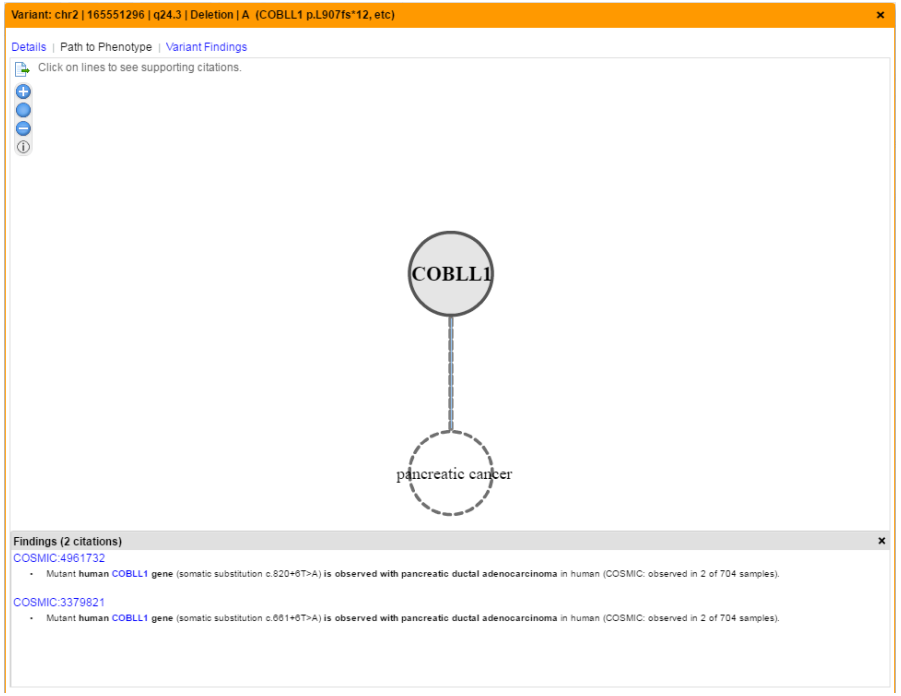
GO Annotations

Molecular Function:	actin monomer binding
Biological Process:	actin filament network formation; actin filament polymerization
Cellular Component:	cell-cell adherens junction; extracellular vesicular exosome

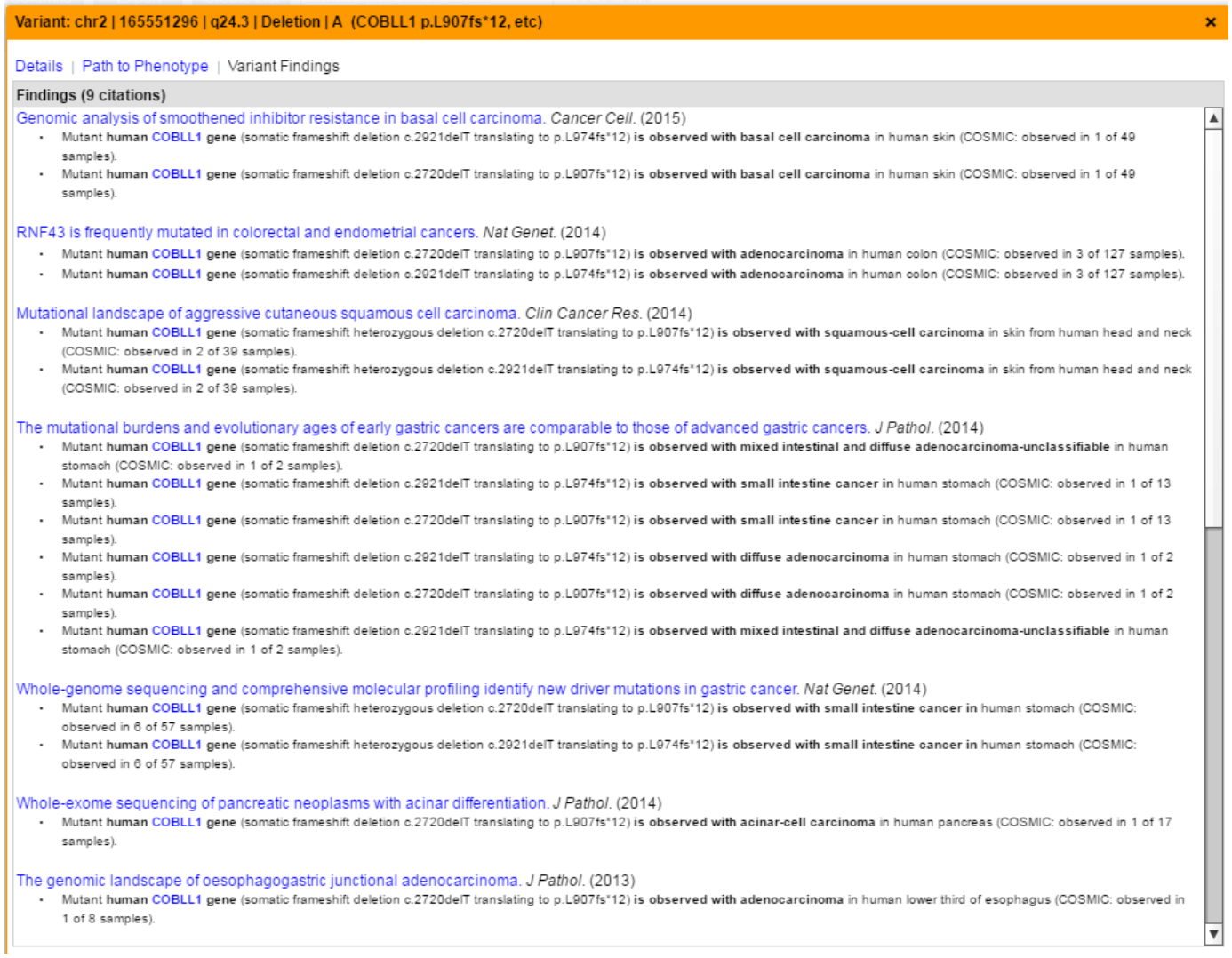
As well as filtering IVA provides a wealth of information…

Coding Effects

Gene Symbol	Region	Transcript ID	Transcript Variant	Protein Variant	Translation Impact	SI
COBLL1	Exonic	NM_001278458.1	c.2921delT	p.L974fs*12	frameshift	
COBLL1	Exonic	NM_001278460.1	c.2744delT	p.L915fs*12	frameshift	
COBLL1	Exonic	NM_001278461.1	c.2606delT	p.L869fs*12	frameshift	
COBLL1	Exonic	NM_014900.4	c.2720delT	p.L907fs*12	frameshift	



As well as filtering IVA provides a wealth of information…



Variant: chr2 | 165551296 | q24.3 | Deletion | A (COBLL1 p.L907fs*12, etc)

Details | Path to Phenotype | Variant Findings

Findings (9 citations)

Genomic analysis of smoothened inhibitor resistance in basal cell carcinoma. *Cancer Cell.* (2015)
- Mutant **human COBLL1 gene** (somatic frameshift deletion c.2921delT translating to p.L974fs*12) **is observed with basal cell carcinoma** in human skin (COSMIC: observed in 1 of 49 samples).
- Mutant **human COBLL1 gene** (somatic frameshift deletion c.2720delT translating to p.L907fs*12) **is observed with basal cell carcinoma** in human skin (COSMIC: observed in 1 of 49 samples).

RNF43 is frequently mutated in colorectal and endometrial cancers. *Nat Genet.* (2014)
- Mutant **human COBLL1 gene** (somatic frameshift deletion c.2720delT translating to p.L907fs*12) **is observed with adenocarcinoma** in human colon (COSMIC: observed in 3 of 127 samples).
- Mutant **human COBLL1 gene** (somatic frameshift deletion c.2921delT translating to p.L974fs*12) **is observed with adenocarcinoma** in human colon (COSMIC: observed in 3 of 127 samples).

Mutational landscape of aggressive cutaneous squamous cell carcinoma. *Clin Cancer Res.* (2014)
- Mutant **human COBLL1 gene** (somatic frameshift heterozygous deletion c.2720delT translating to p.L907fs*12) **is observed with squamous-cell carcinoma** in skin from human head and neck (COSMIC: observed in 2 of 39 samples).
- Mutant **human COBLL1 gene** (somatic frameshift heterozygous deletion c.2921delT translating to p.L974fs*12) **is observed with squamous-cell carcinoma** in skin from human head and neck (COSMIC: observed in 2 of 39 samples).

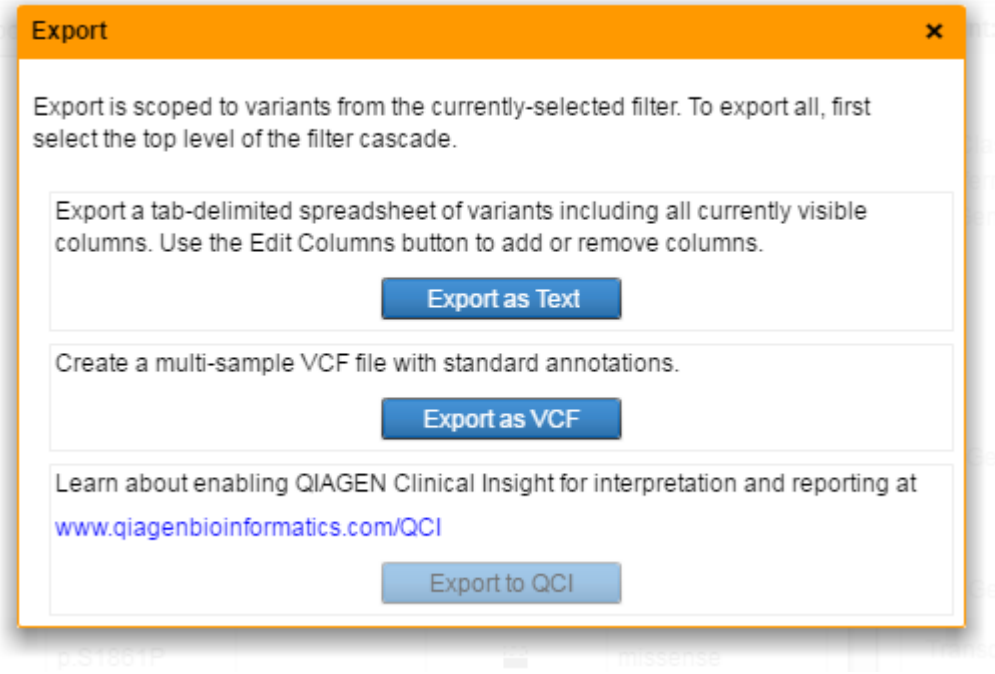
The mutational burdens and evolutionary ages of early gastric cancers are comparable to those of advanced gastric cancers. *J Pathol.* (2014)
- Mutant **human COBLL1 gene** (somatic frameshift deletion c.2720delT translating to p.L907fs*12) **is observed with mixed intestinal and diffuse adenocarcinoma-unclassifiable** in human stomach (COSMIC: observed in 1 of 2 samples).
- Mutant **human COBLL1 gene** (somatic frameshift deletion c.2921delT translating to p.L974fs*12) **is observed with small intestine cancer in** human stomach (COSMIC: observed in 1 of 13 samples).
- Mutant **human COBLL1 gene** (somatic frameshift deletion c.2720delT translating to p.L907fs*12) **is observed with small intestine cancer in** human stomach (COSMIC: observed in 1 of 13 samples).
- Mutant **human COBLL1 gene** (somatic frameshift deletion c.2921delT translating to p.L974fs*12) **is observed with diffuse adenocarcinoma** in human stomach (COSMIC: observed in 1 of 2 samples).
- Mutant **human COBLL1 gene** (somatic frameshift deletion c.2720delT translating to p.L907fs*12) **is observed with diffuse adenocarcinoma** in human stomach (COSMIC: observed in 1 of 2 samples).
- Mutant **human COBLL1 gene** (somatic frameshift deletion c.2921delT translating to p.L974fs*12) **is observed with mixed intestinal and diffuse adenocarcinoma-unclassifiable** in human stomach (COSMIC: observed in 1 of 2 samples).

Whole-genome sequencing and comprehensive molecular profiling identify new driver mutations in gastric cancer. *Nat Genet.* (2014)
- Mutant **human COBLL1 gene** (somatic frameshift heterozygous deletion c.2720delT translating to p.L907fs*12) **is observed with small intestine cancer in** human stomach (COSMIC: observed in 6 of 57 samples).
- Mutant **human COBLL1 gene** (somatic frameshift heterozygous deletion c.2921delT translating to p.L974fs*12) **is observed with small intestine cancer in** human stomach (COSMIC: observed in 6 of 57 samples).

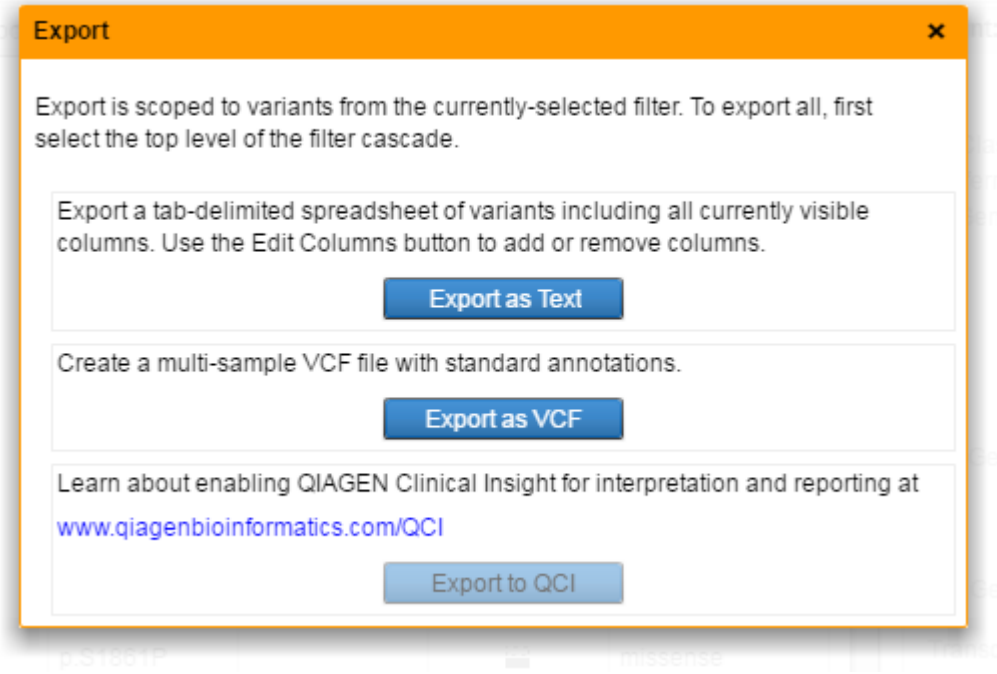
Whole-exome sequencing of pancreatic neoplasms with acinar differentiation. *J Pathol.* (2014)
- Mutant **human COBLL1 gene** (somatic frameshift deletion c.2720delT translating to p.L907fs*12) **is observed with acinar-cell carcinoma** in human pancreas (COSMIC: observed in 1 of 17 samples).

The genomic landscape of oesophagogastric junctional adenocarcinoma. *J Pathol.* (2013)
- Mutant **human COBLL1 gene** (somatic frameshift deletion c.2720delT translating to p.L907fs*12) **is observed with adenocarcinoma** in human lower third of esophagus (COSMIC: observed in 1 of 8 samples).

What can you do with your filtered list?

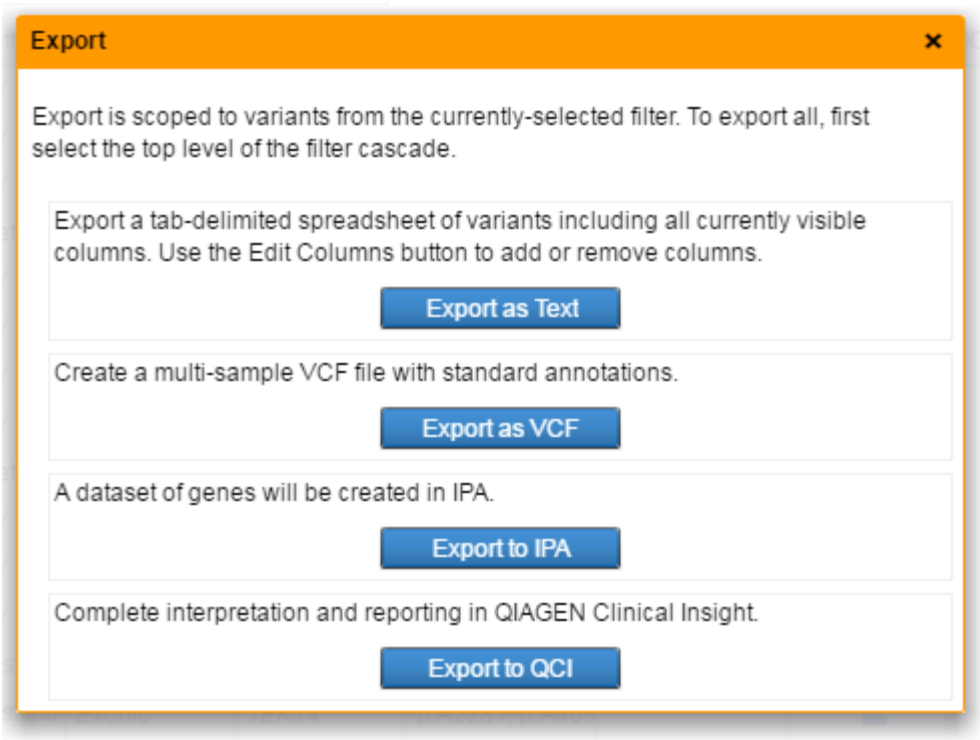


To export to QCI I you need a QCI I account

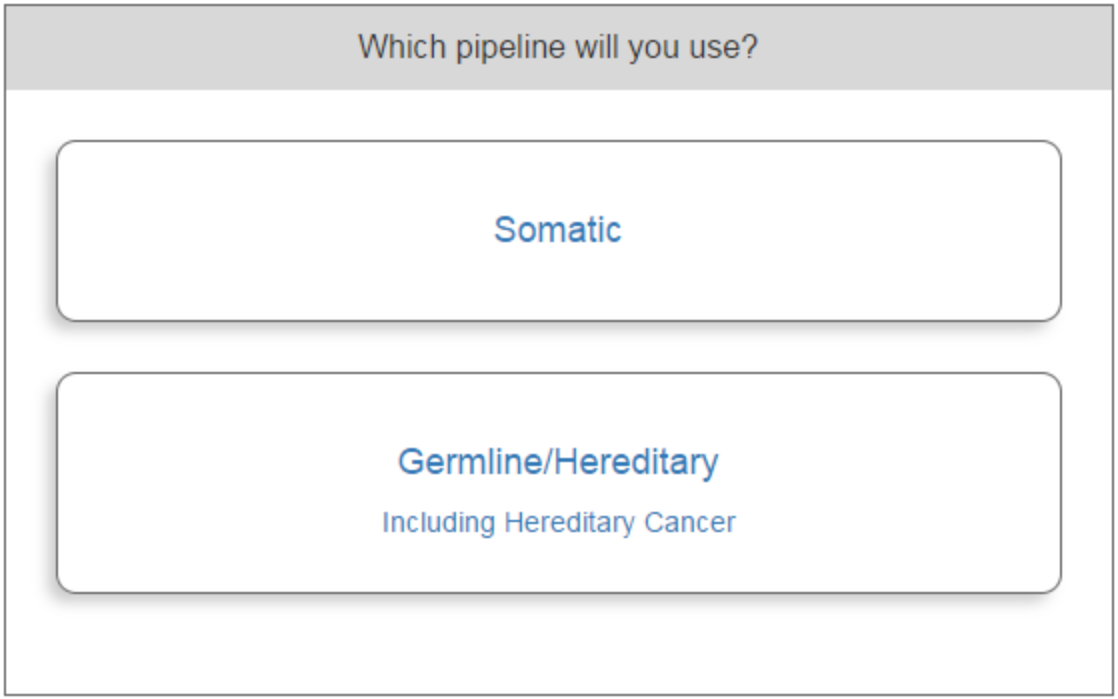


QCI I Demo

To export to QCI I you need a QCI I account



Choose your workflow



Add in patient meta data

Test Product Profile (optional)

Custom specification of default values that remain constant for a given test product (includes reporting method, report template, and more). Consult with our clinical science team to create one or more test product profile(s).

Test Product Code (required)

Your laboratory's unique identifier for the test that was ordered (usually a catalog # or SKU).

Accession ID (required)

TCRBOA2-T-WEX.read1 (paired) Read Mapping (Locally Realigned, Variants, MVF), TCRBOA2-N-

Your laboratory's unique identifier for the sample.

Test Date (required)

YYYY-MM-DD

Date that this accession entered your system.

Diagnosis (required)

This is used to match treatments and trials and for display on the report.

Diagnosis Stage (optional)

<unselected>

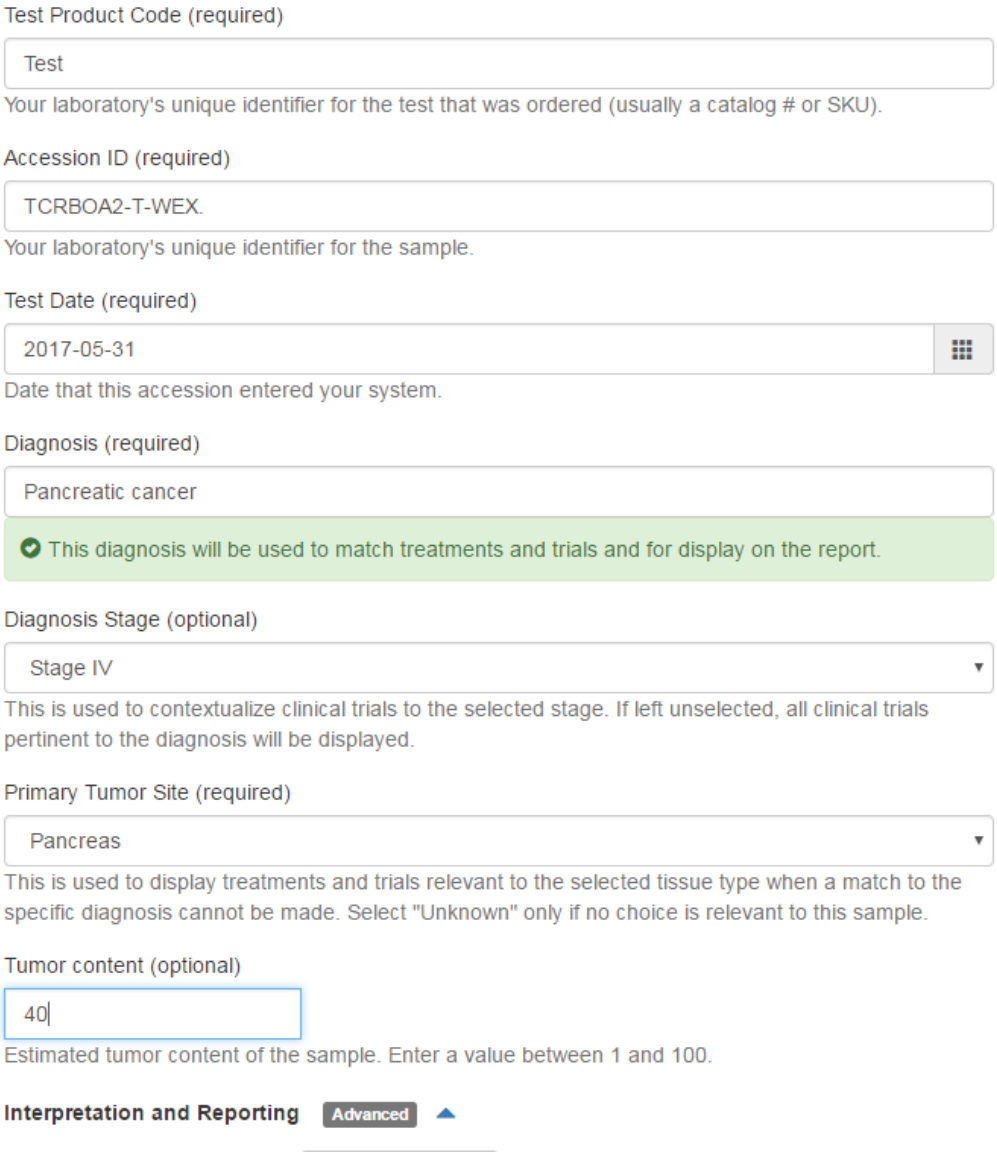
This is used to contextualize clinical trials to the selected stage. If left unselected, all clinical trials pertinent to the diagnosis will be displayed.

Primary Tumor Site (required)

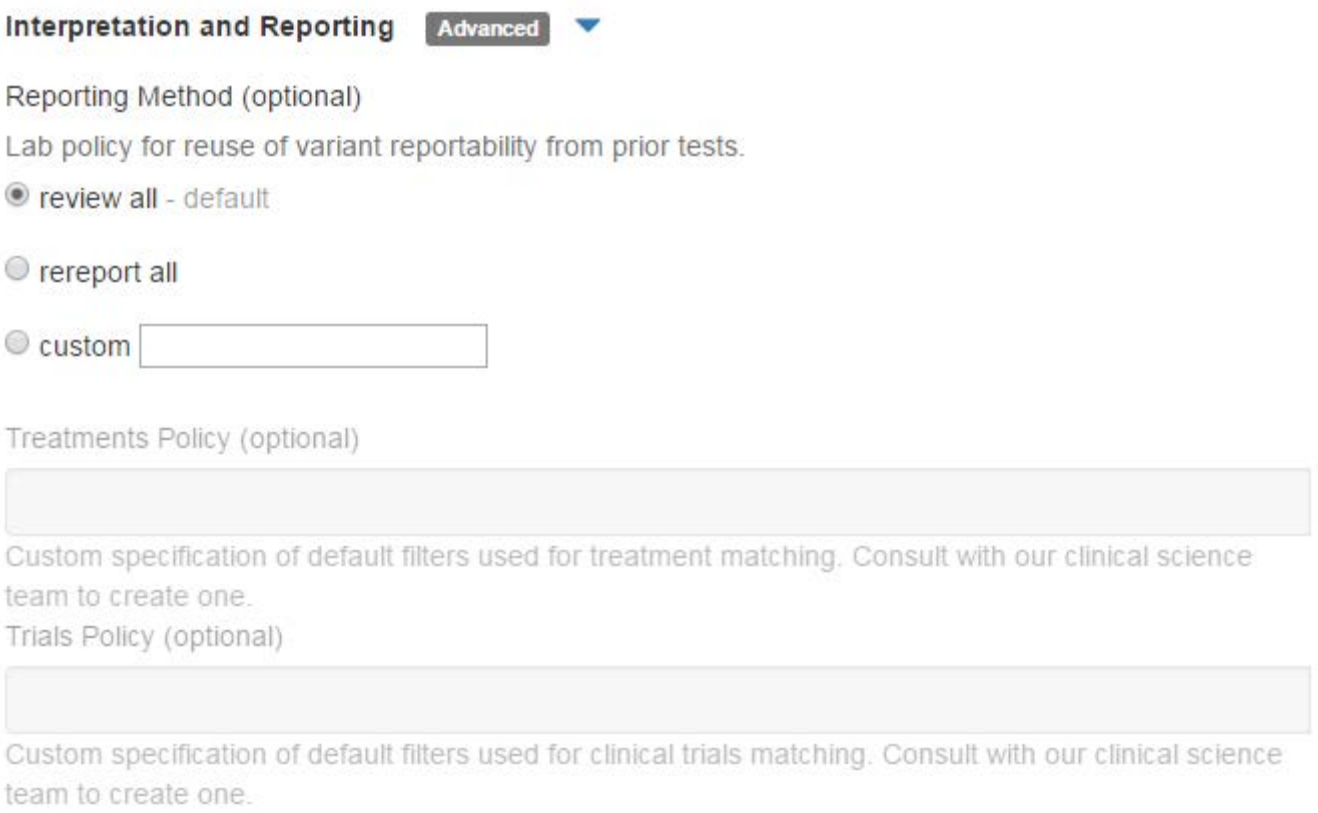
<unselected>

This is used to display treatments and trials relevant to the selected tissue type when a match to the specific diagnosis cannot be made. Select "Unknown" only if no choice is relevant to this sample.

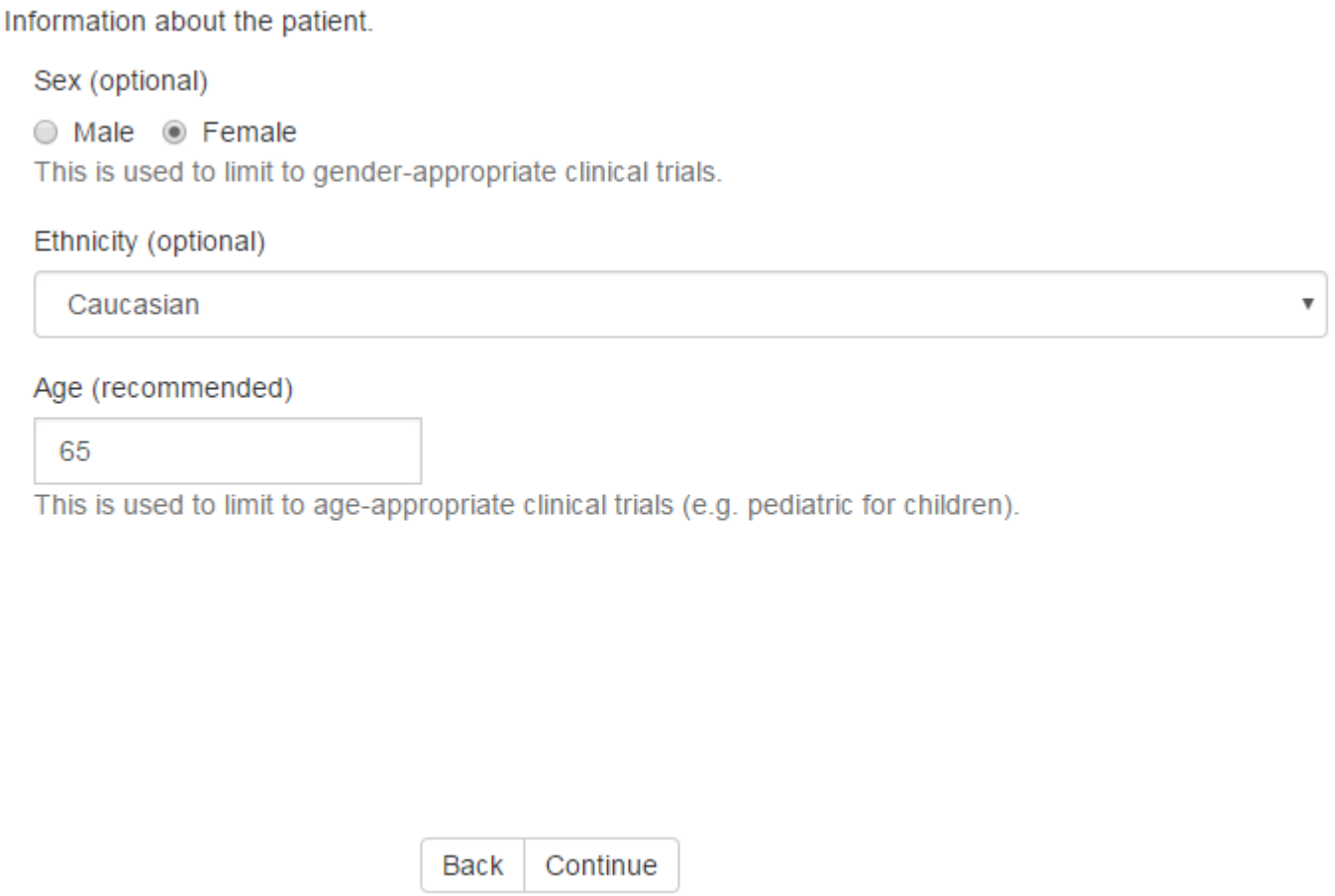
Add in patient meta data



The software will “learn” from your classifications…



Further meta data…



Further meta data…

The information below is used only on the final report. For validation samples, you can use the data that has been prefilled for you below, or hit Clear Data and enter your own information.

Demo Data | Clear Data | Reset Data

Patient Name (optional)
Michelle Doe

Client Name (optional)
General Hospital

Client ID (optional)
ABC123

Physician Name (optional)
Dr. E Smith

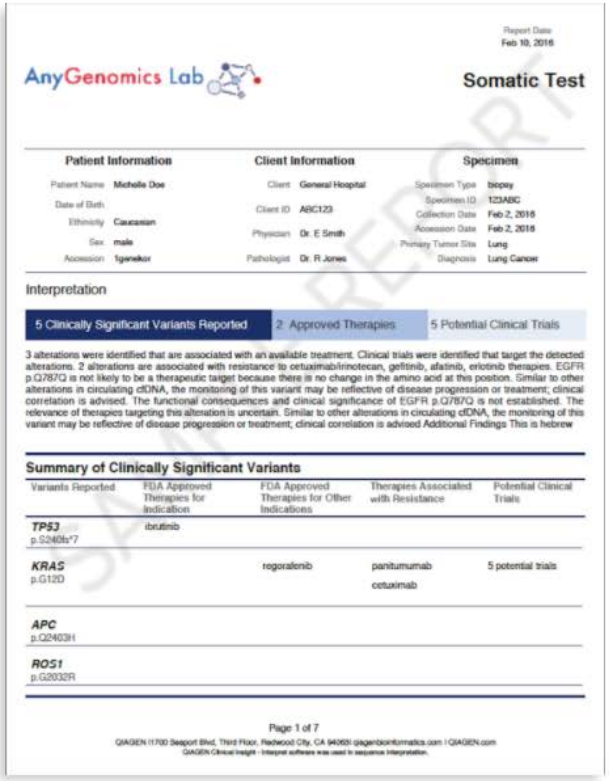
Pathologist Name (optional)
Dr. R Jones

Specimen Type (optional)
biopsy

Specimen ID (optional)
ABC123

Specimen Dissection (optional)
manual

Date of Birth (optional)
May | 31 | 2017
Month | Day | Year


Report Date
Feb 10, 2016

AnyGenomics Lab

Somatic Test

Patient Information		Client Information		Specimen	
Patient Name	Michelle Doe	Client	General Hospital	Specimen Type	biopsy
Date of Birth		Client ID	ABC123	Specimen ID	123ABC
Ethnicity	Caucasian	Physician	Dr. E Smith	Collection Date	Feb 2, 2016
Sex	male	Pathologist	Dr. R Jones	Accession Date	Feb 2, 2016
Accession	1genekor			Primary Tumor Site	Lung
				Diagnosis	Lung Cancer

Interpretation

5 Clinically Significant Variants Reported | 2 Approved Therapies | 5 Potential Clinical Trials

3 alterations were identified that are associated with an available treatment. Clinical trials were identified that target the detected alterations. 2 alterations are associated with resistance to cetuximab/irinotecan, gefitinib, afatinib, erlotinib therapies. EGFR p.Q787Q is not likely to be a therapeutic target because there is no change in the amino acid at this position. Similar to other alterations in circulating cfDNA, the monitoring of this variant may be reflective of disease progression or treatment; clinical correlation is advised. The functional consequences and clinical significance of EGFR p.Q787Q is not established. The relevance of therapies targeting this alteration is uncertain. Similar to other alterations in circulating cfDNA, the monitoring of this variant may be reflective of disease progression or treatment; clinical correlation is advised Additional Findings This is hebrew

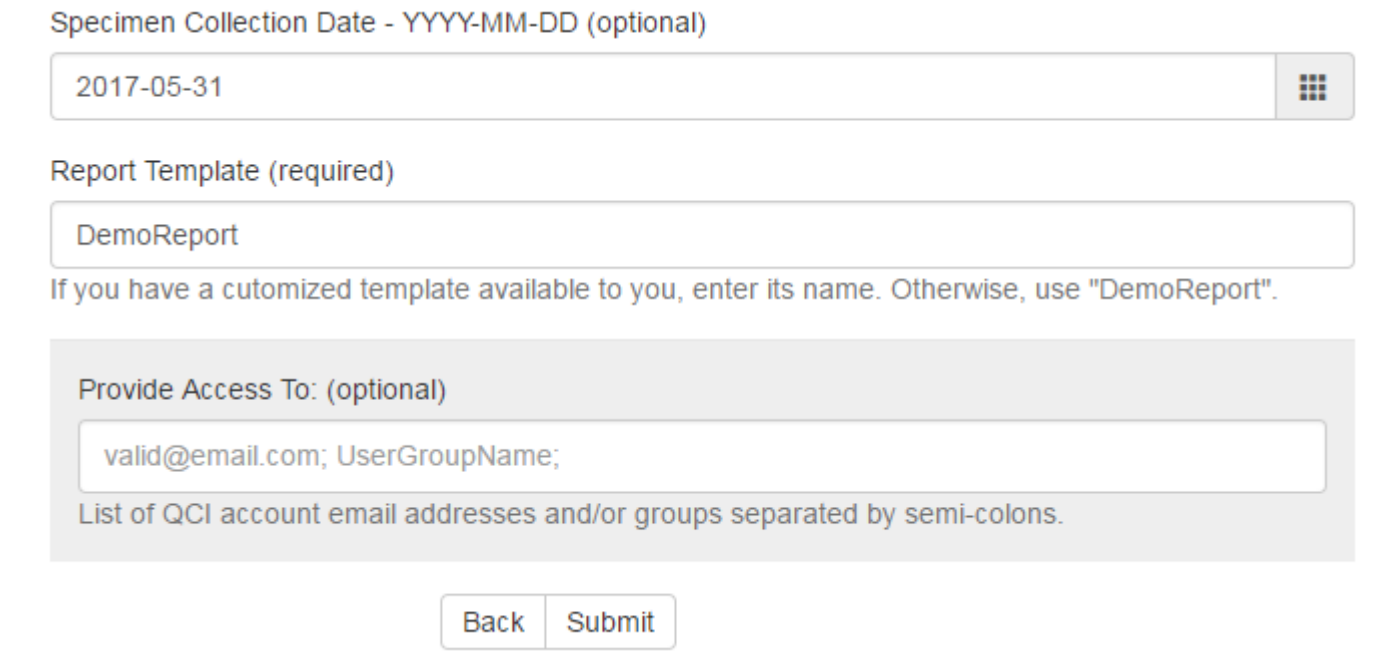
Summary of Clinically Significant Variants

Variants Reported	FDA Approved Therapies for Indication	FDA Approved Therapies for Other Indications	Therapies Associated with Resistance	Potential Clinical Trials
TP53 p.S240fs*7	ibrutinib			
KRAS p.G12D		regorafenib	panitumumab cetuximab	5 potential trials
APC p.Q2403H				
ROS1 p.G2032R				

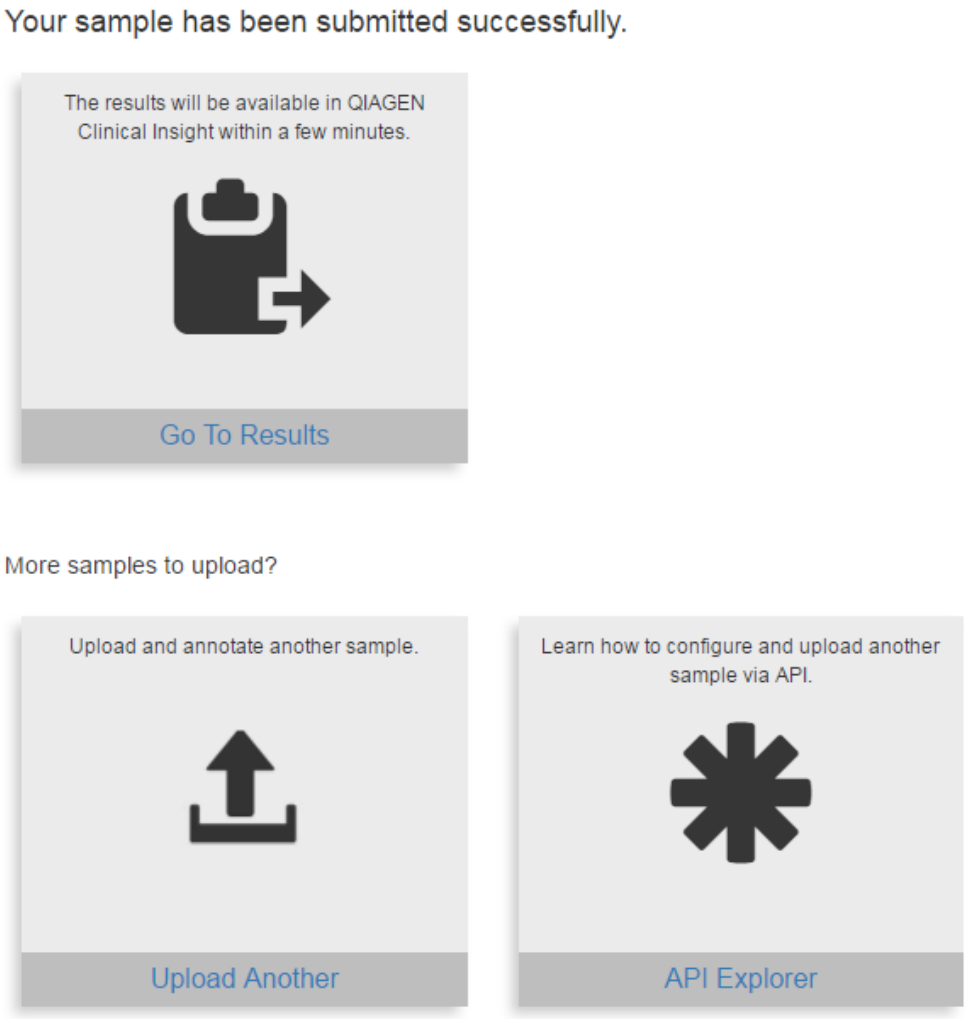
Page 1 of 7

QIAGEN 11700 Seaport Blvd, Third Floor, Redwood City, CA 94065| qiagenbioinformatics.com | QIAGEN.com
QIAGEN Clinical Insight - Interpret software was used in sequence interpretation.

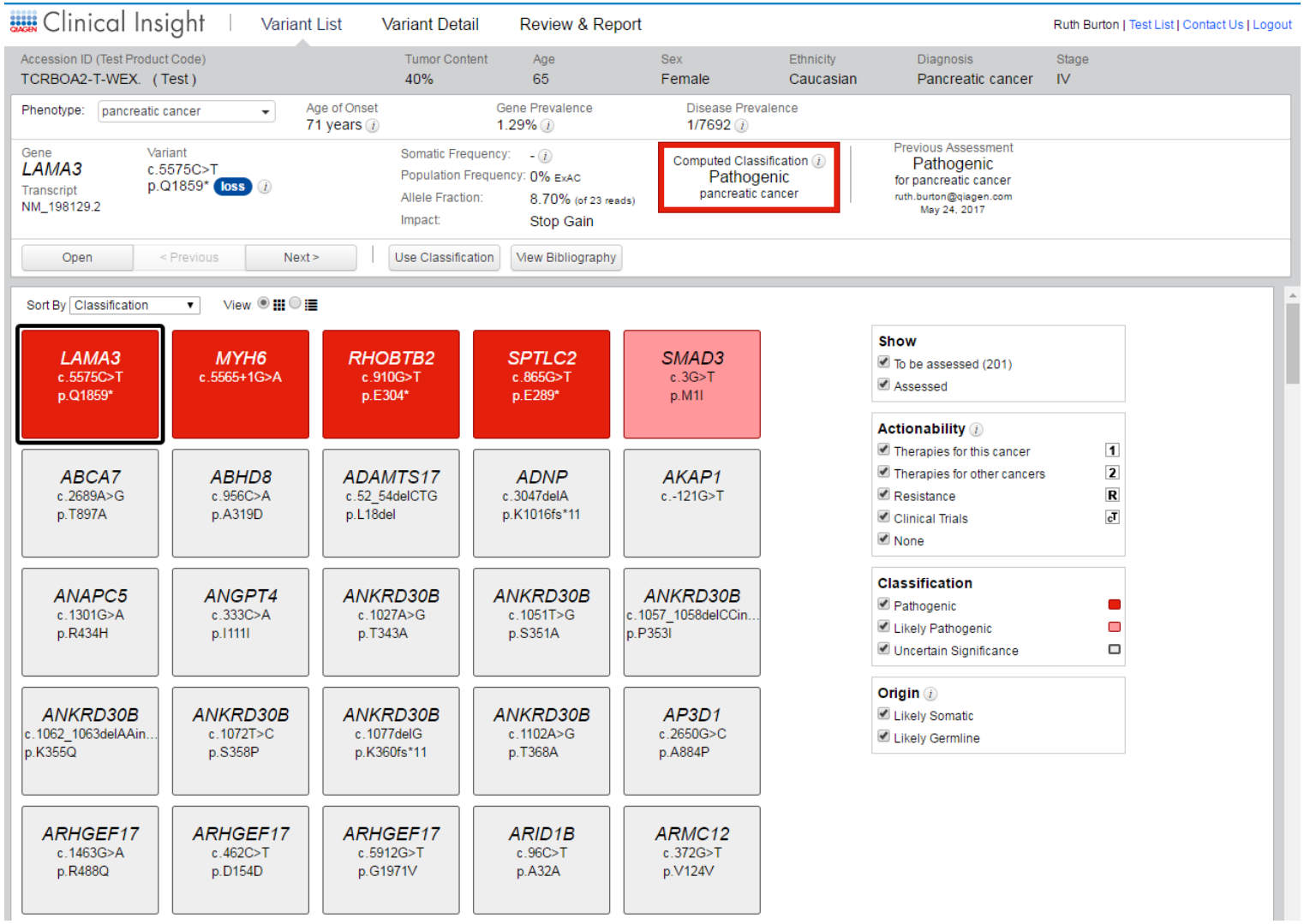
Option for customized report…



Go to results…

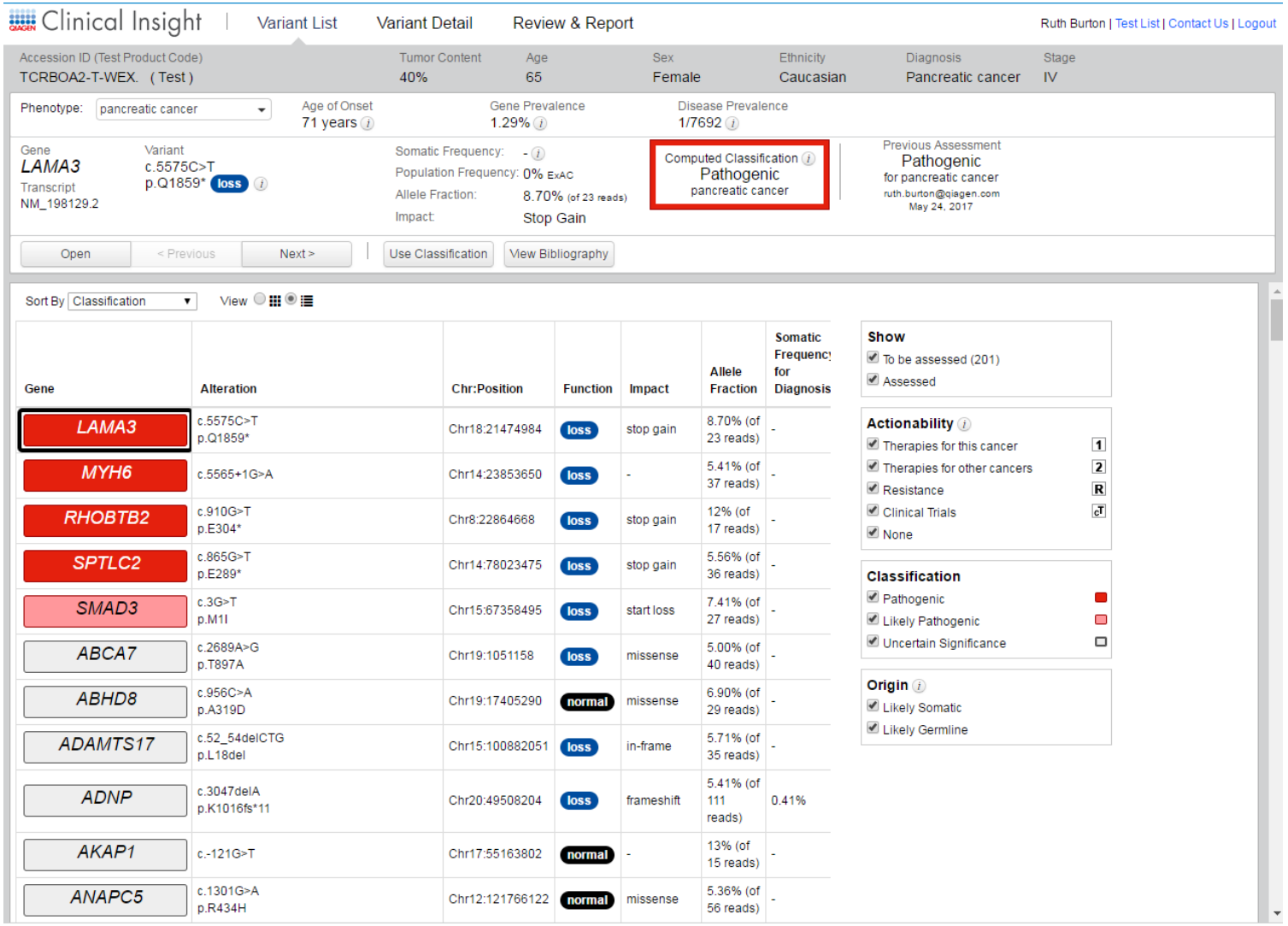


The list of variants…

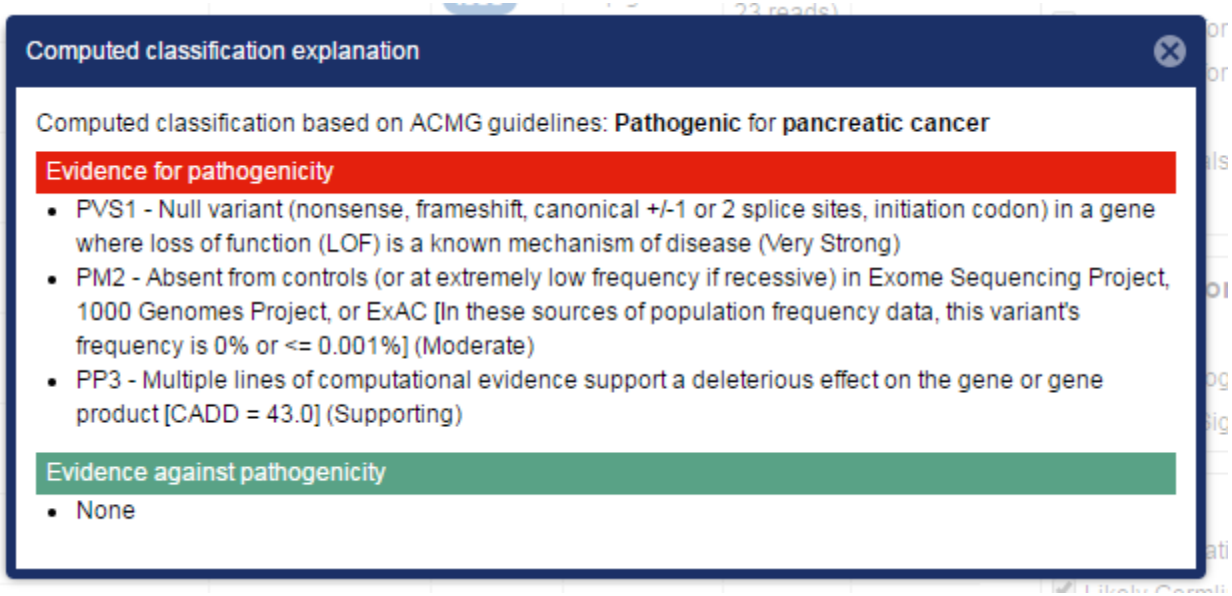


QCI I Demo

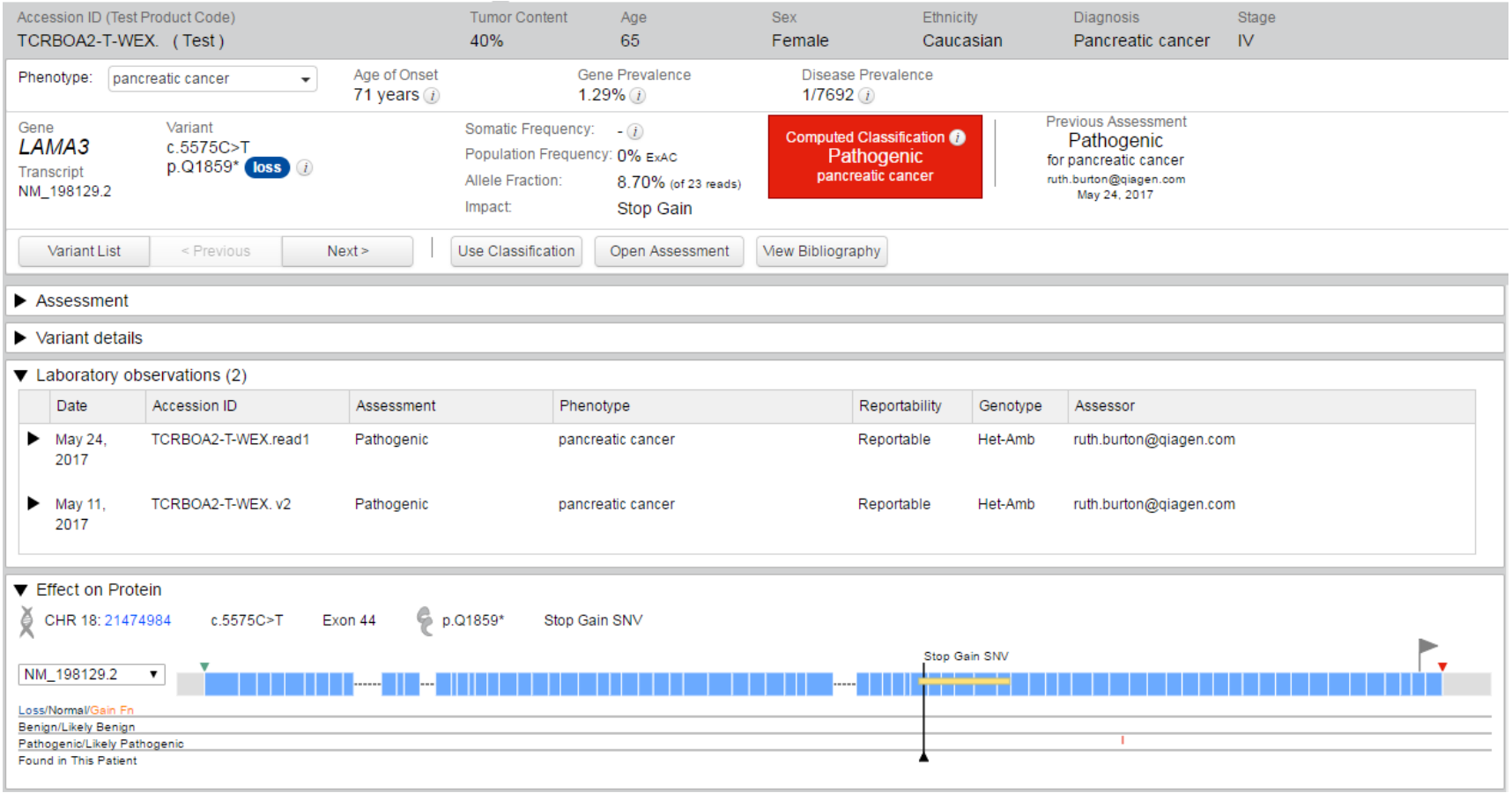
Two views… a lot of information is already given about the variant



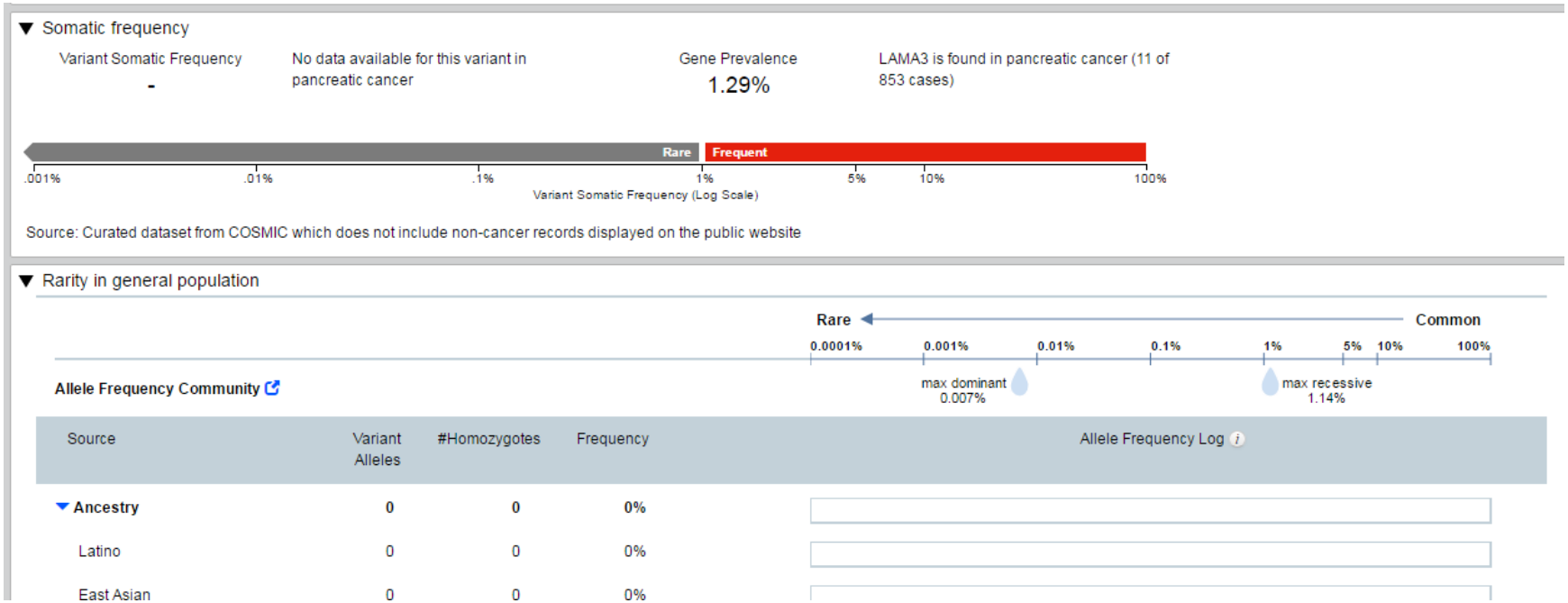
ACMG Guidelines are used for classification



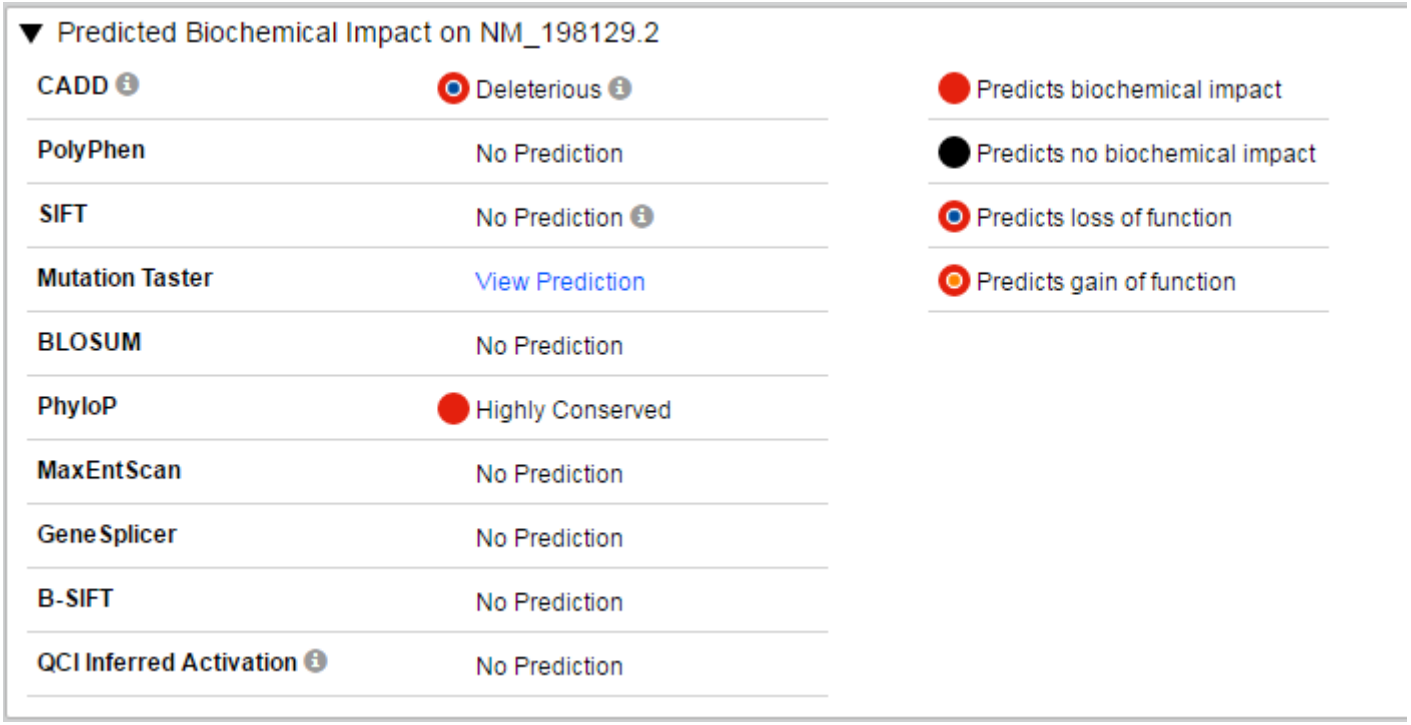
Variant details provide supporting evidence for classification



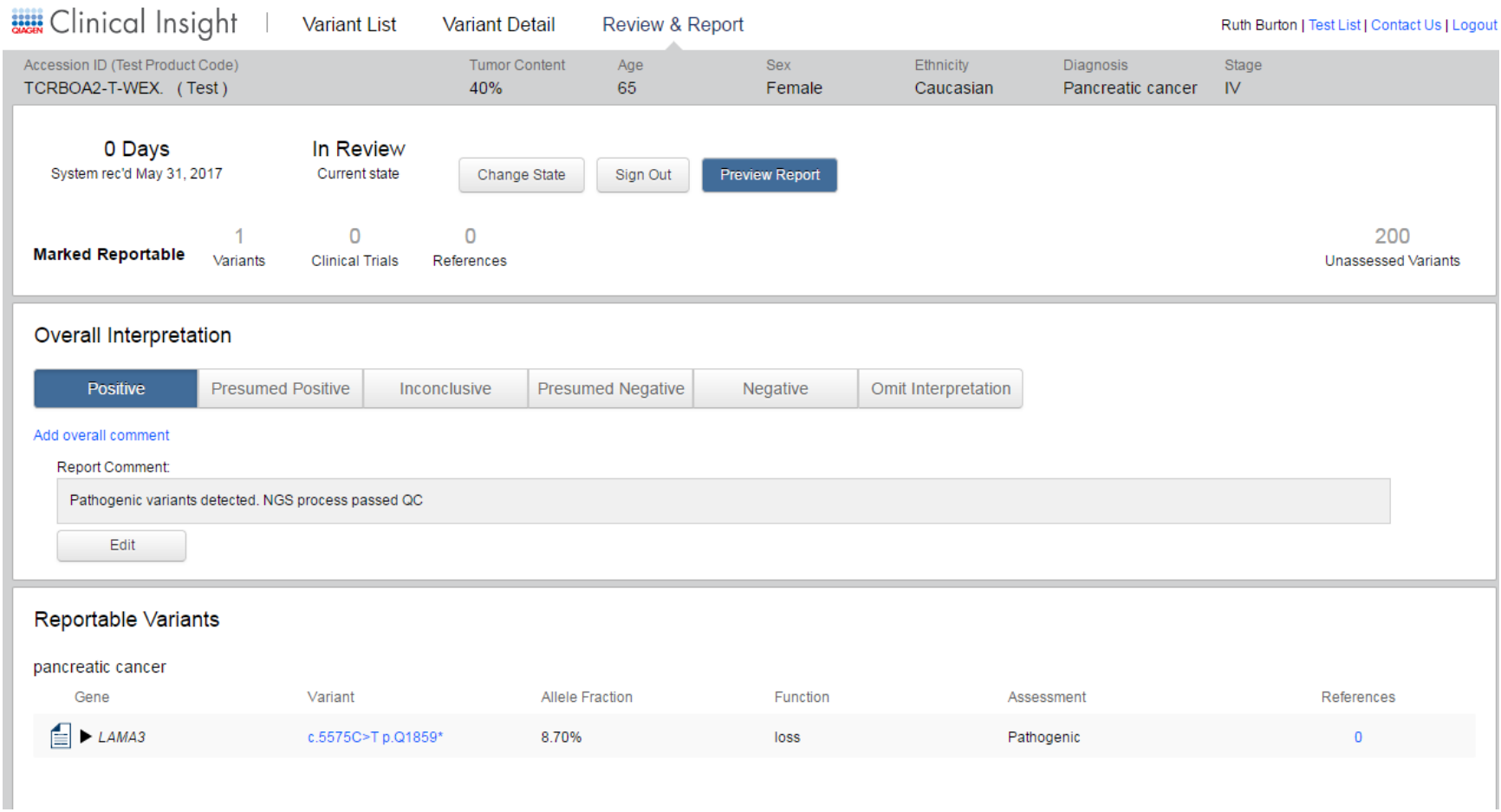
Variant details provide supporting evidence for classification



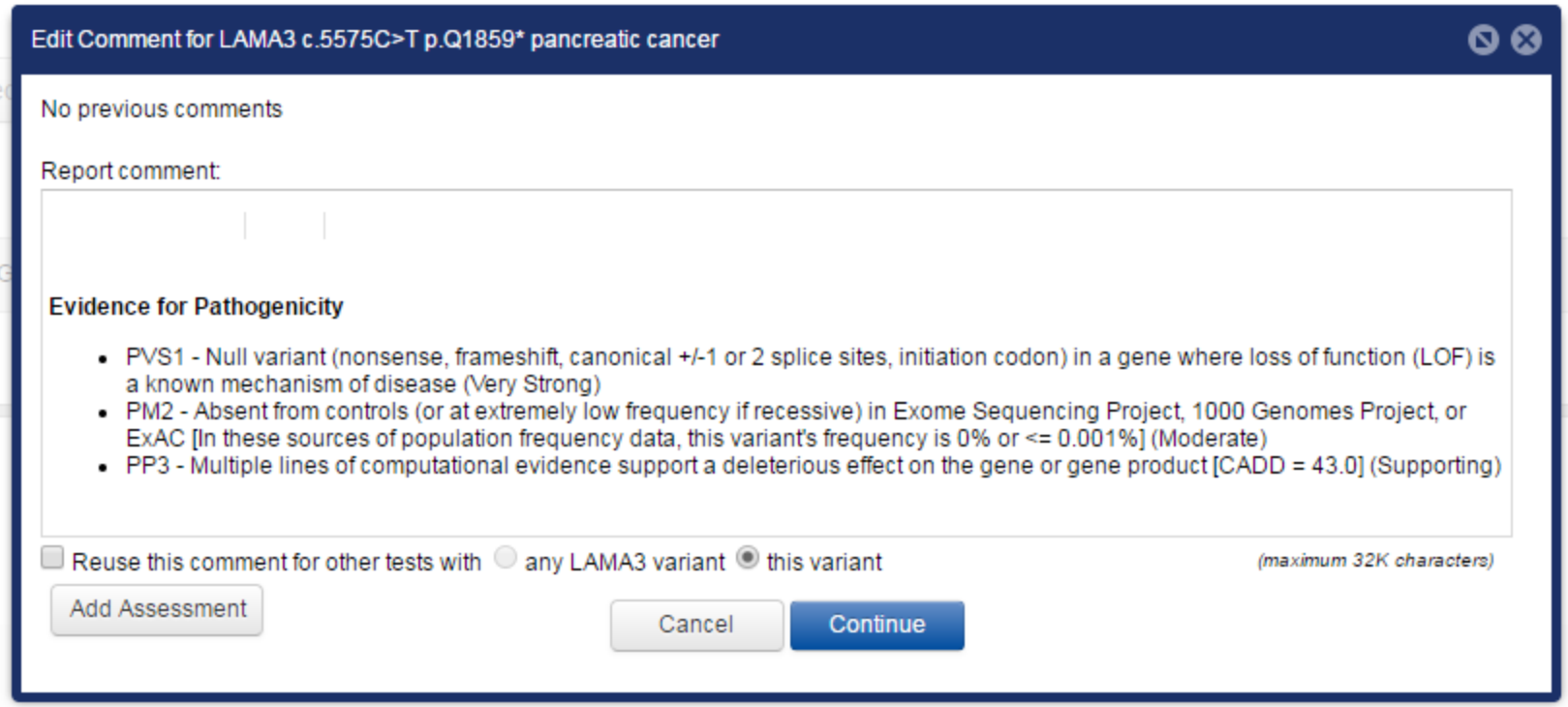
Variant details provide supporting evidence for classification



Key step is creating a report



Key step is creating a report



Key step is creating a report

Somatic Test

AnyGenomics Lab

Report Date May 31, 2017
Report Status -

Patient Information

Patient Name	Michelle Doe
Date of Birth	May 31, 2017
Ethnicity	Caucasian
Sex	female
Accession	TCRBOA2-T-WEX.

Client Information

Client	General Hospital
Client ID	ABC123
Physician	Dr. E Smith
Pathologist	Dr. R Jones

Specimen

Specimen Type	biopsy
Specimen ID	ABC123
Collection Date	May 31, 2017
Accession Date	May 31, 2017
Primary Tumor Site	Pancreas
Diagnosis	Pancreatic cancer
Diagnosis Stage	IV

Interpretation

1 Clinically Significant Variant Reported | 0 Approved Therapy | 0 Potential Clinical Trials

Pathogenic variants detected. NGS process passed QC

Summary of Clinically Significant Variants

Variants Reported	Allele Fraction	Approved Therapies for Same Cancer	Approved Therapies for Other Cancers	Therapies Associated with Resistance	Potential Clinical Trials
LAMA3 p.Q1859*	8.7% (of 23 reads)				

Variant Details

Gene	Exon #	Nucleotide Change	Amino Acid Change	Effect on Protein
LAMA3	44	NM_198129.2: c.5575C>T	p.Q1859*	loss of function

Evidence for Pathogenicity

- PVS1 - Null variant (nonsense, frameshift, canonical +/-1 or 2 splice sites, initiation codon) in a gene where loss of function (LOF) is a known mechanism of disease (Very Strong)
- PM2 - Absent from controls (or at extremely low frequency if recessive) in Exome Sequencing Project, 1000 Genomes Project, or ExAC [In these sources of population frequency data, this variant's frequency is 0% or <= 0.001%] (Moderate)
- PP3 - Multiple lines of computational evidence support a deleterious effect on the gene or gene product [CADD = 43.0] (Supporting)

Genes Tested

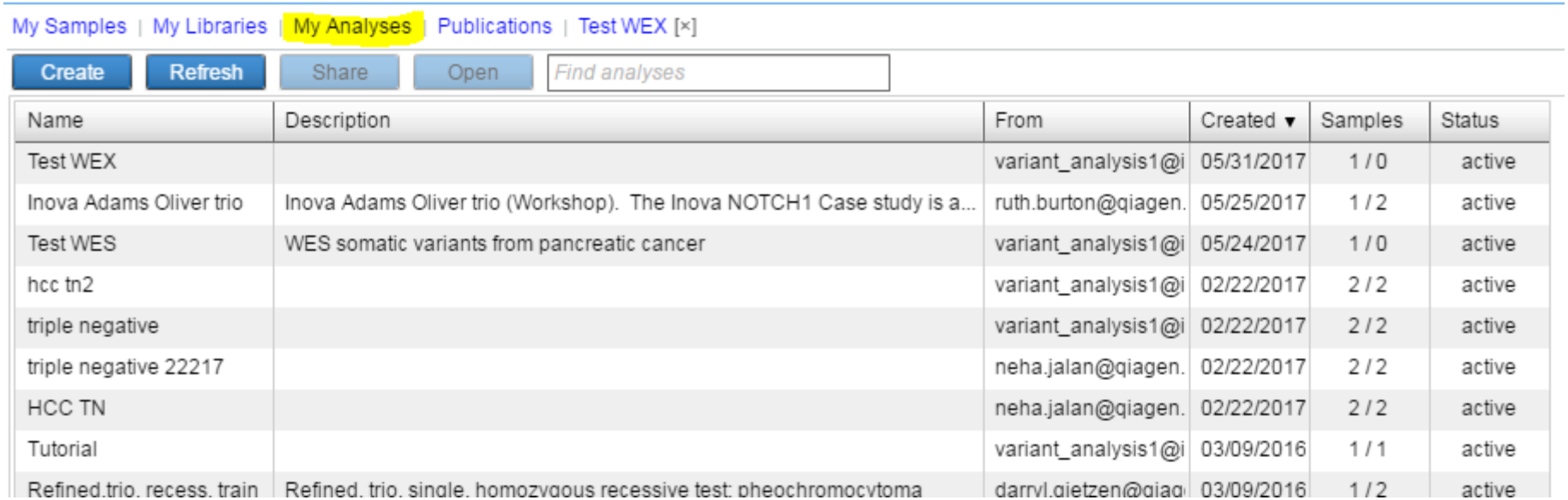
KRAS NRAS KIT BRAF PDGFRA ALK EGFR ERBB2 PIK3CA ERBB3 ESR1 RAF1

Methods and Limitations

EXAMPLE Statement including sample type (FFPE, etc), method of extraction, amplification reactions, panel targeted regions, sequencing technology, etc. Additionally, a description of the data analysis software(s), genome of reference and the sensitivity of the methods should be described.

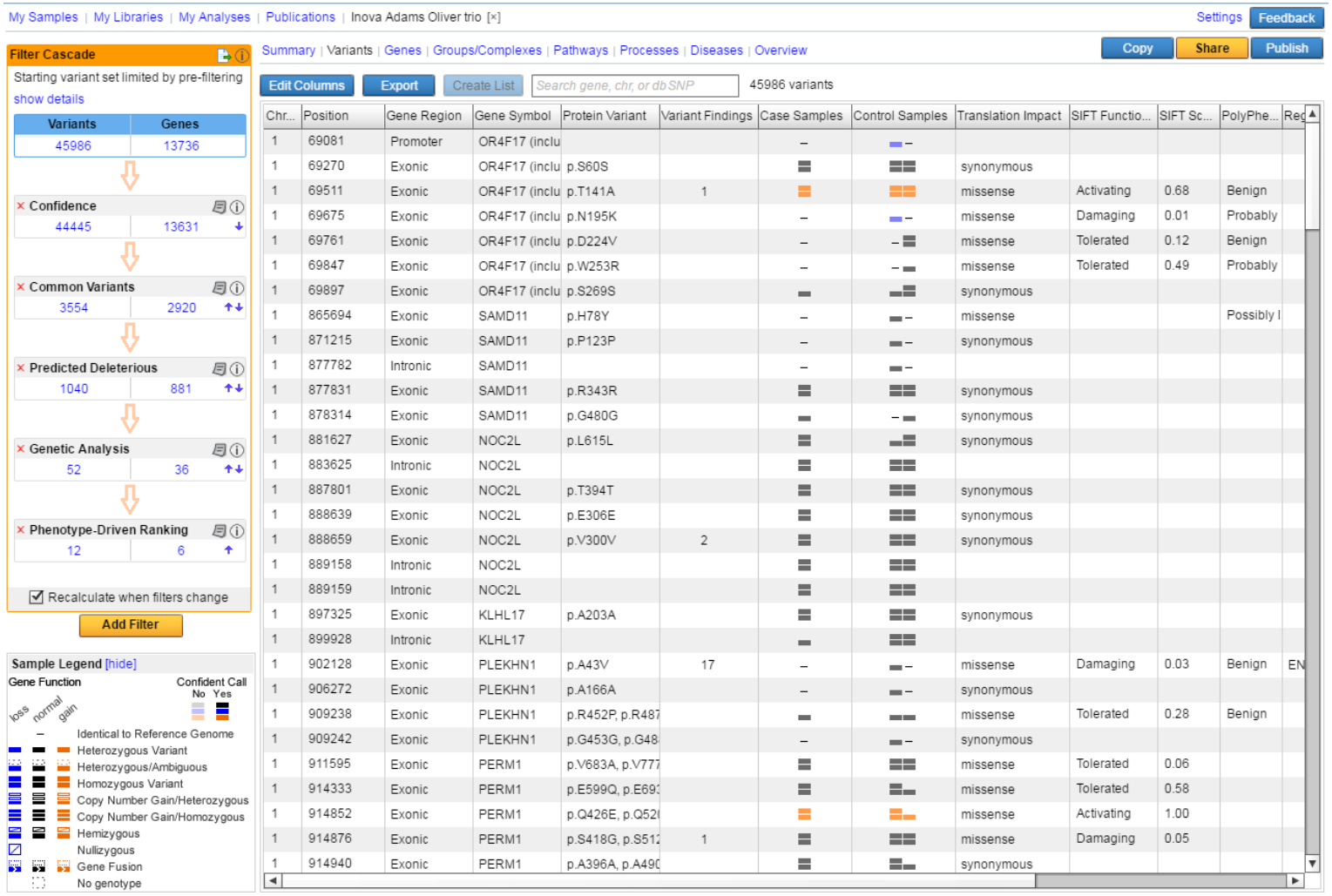
QIAGEN Clinical Insight - Interpret software was used in sequence analysis and interpretation. The application was internally designed and developed by QIAGEN. All analyses were based on: QIAGEN Clinical Insight-Interpret (4.4.20170525), Ingenuity Knowledge Base (Lorien 170520.000), CADD (v1.3), EVS (ESP6500SI-V2), Allele Frequency Community (2017-01-31), JASPAR (2013-11), Vista Enhancer hg18 (2012-07), Vista Enhancer hg19 (2012-07), Clinical Trials (Lorien 170520.000), BSIFT (2016-02-23), TCGA (2013-09-05), PolyPhen-2 (v2.2.2), 1000 Genome Frequency (phase3v5b), Clinvar (2017-01-04), DGV (2016-05-15), COSMIC (v79), ExAC (0.3.1), HGMD (2016.4), PhyloP hg18 (2009-11), PhyloP hg19 (2009-11), DbSNP (149), TargetScan (6.2), SIFT4G (2016-02-23) Weekly updates to Ingenuity Knowledge Base for clinical trials recruitment status and new findings from recent articles.Variants are reported according to HGVS nomenclature and were classified following ACMG guidelines. Information on therapeutic agents and clinical trials were obtained from publicly available information. Variants, therapies, and trials listed in this report are not ranked in order of potential clinical significance or predicted efficacy for this patient.

Trio Analysis, highlight and click “Open”

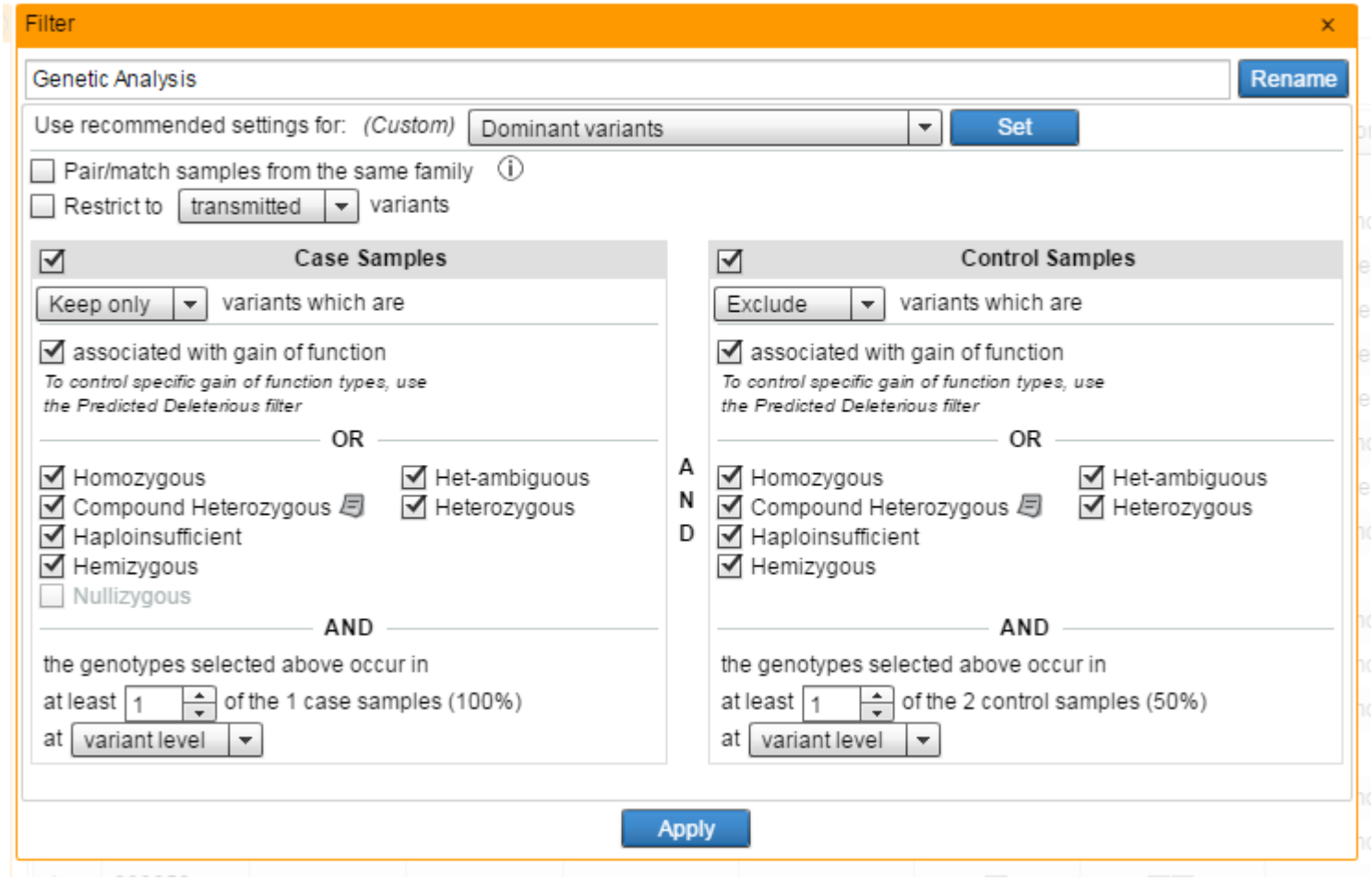


Name	Description	From	Created ▾	Samples	Status
Test WEX		variant_analysis1@i	05/31/2017	1 / 0	active
Inova Adams Oliver trio	Inova Adams Oliver trio (Workshop). The Inova NOTCH1 Case study is a...	ruth.burton@qiagen.	05/25/2017	1 / 2	active
Test WES	WES somatic variants from pancreatic cancer	variant_analysis1@i	05/24/2017	1 / 0	active
hcc tn2		variant_analysis1@i	02/22/2017	2 / 2	active
triple negative		variant_analysis1@i	02/22/2017	2 / 2	active
triple negative 22217		neha.jalan@qiagen.	02/22/2017	2 / 2	active
HCC TN		neha.jalan@qiagen.	02/22/2017	2 / 2	active
Tutorial		variant_analysis1@i	03/09/2016	1 / 1	active
Refined.trio. recess. train	Refined. trio. single. homozygous recessive test: pheochromocytoma	darryl.gietzen@giag	03/09/2016	1 / 2	active

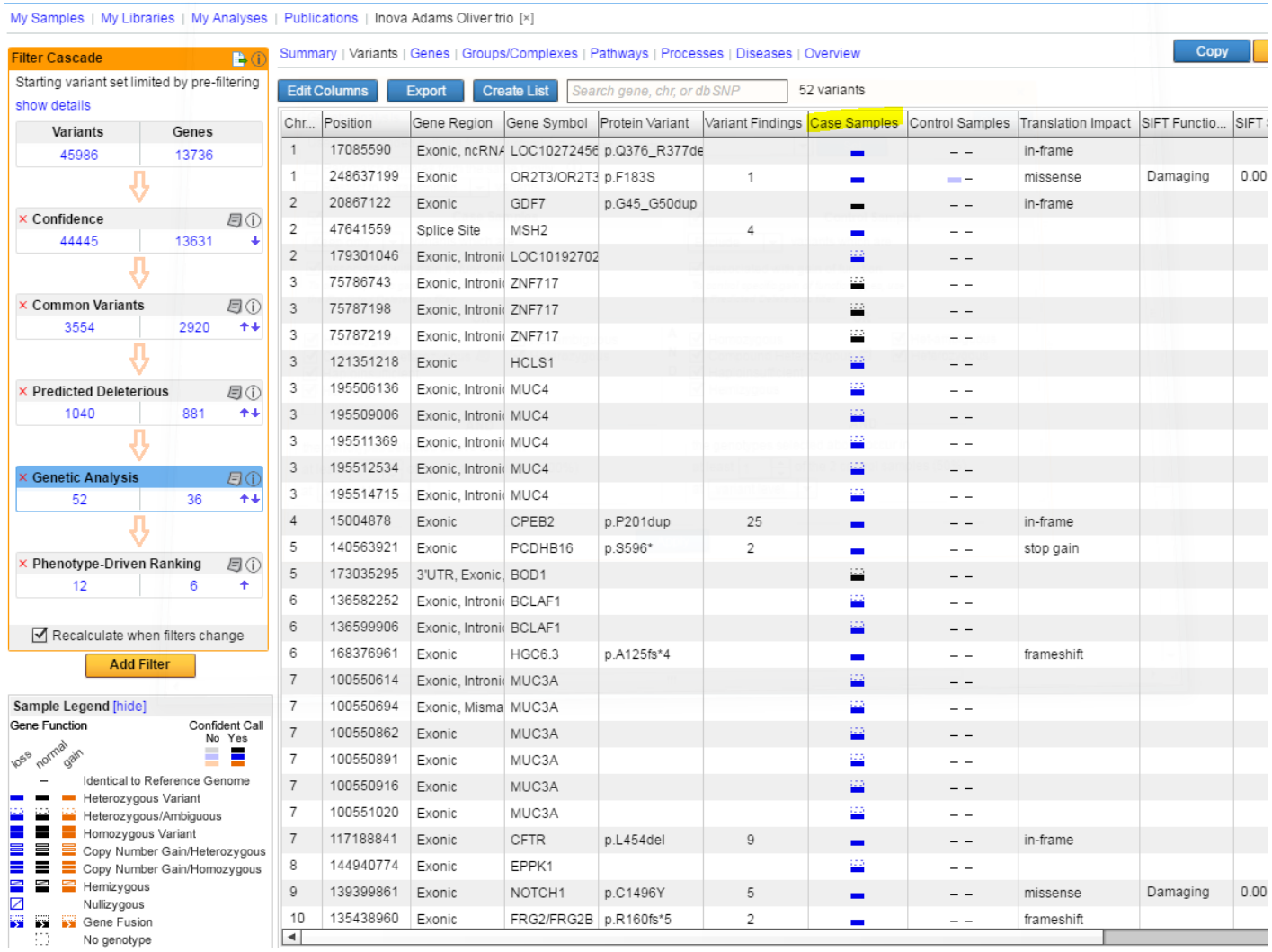
Here we have a different set up and filters, click on the top of the filter cascade…



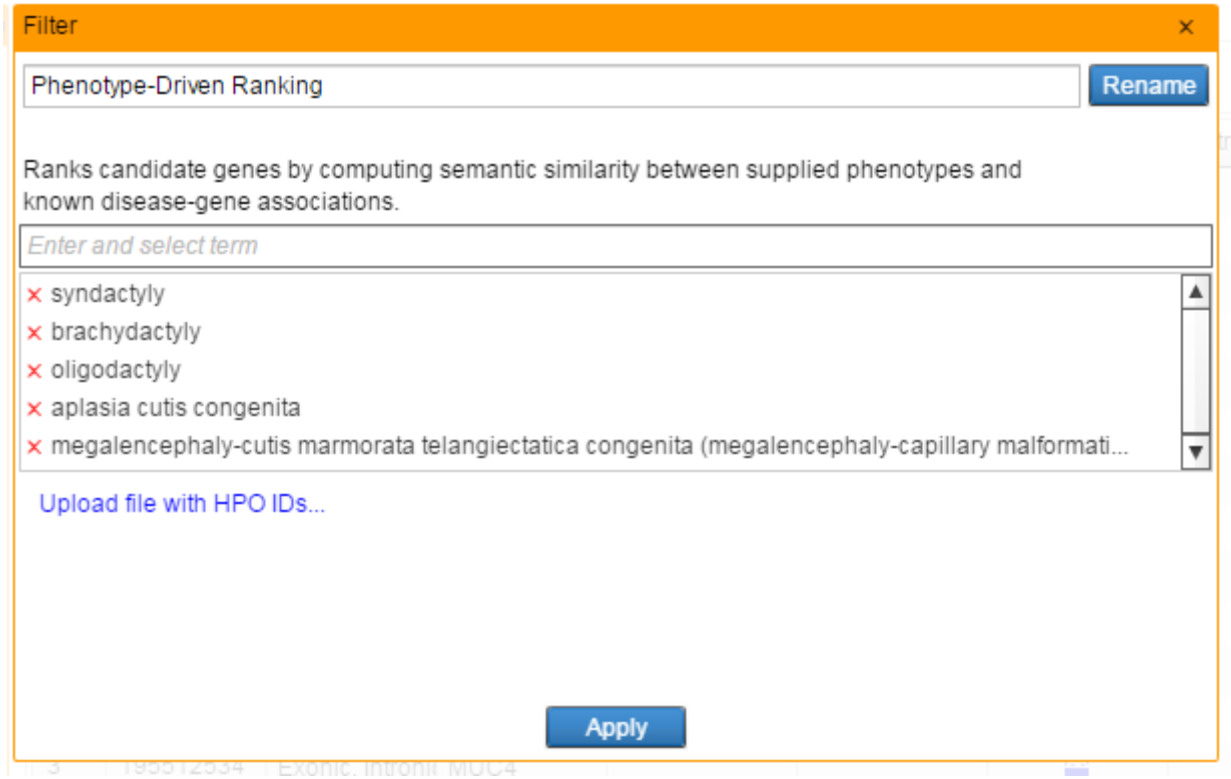
While the same principles are used as with the single sample here we can use the parental samples to detect *de novo* variants in the child…



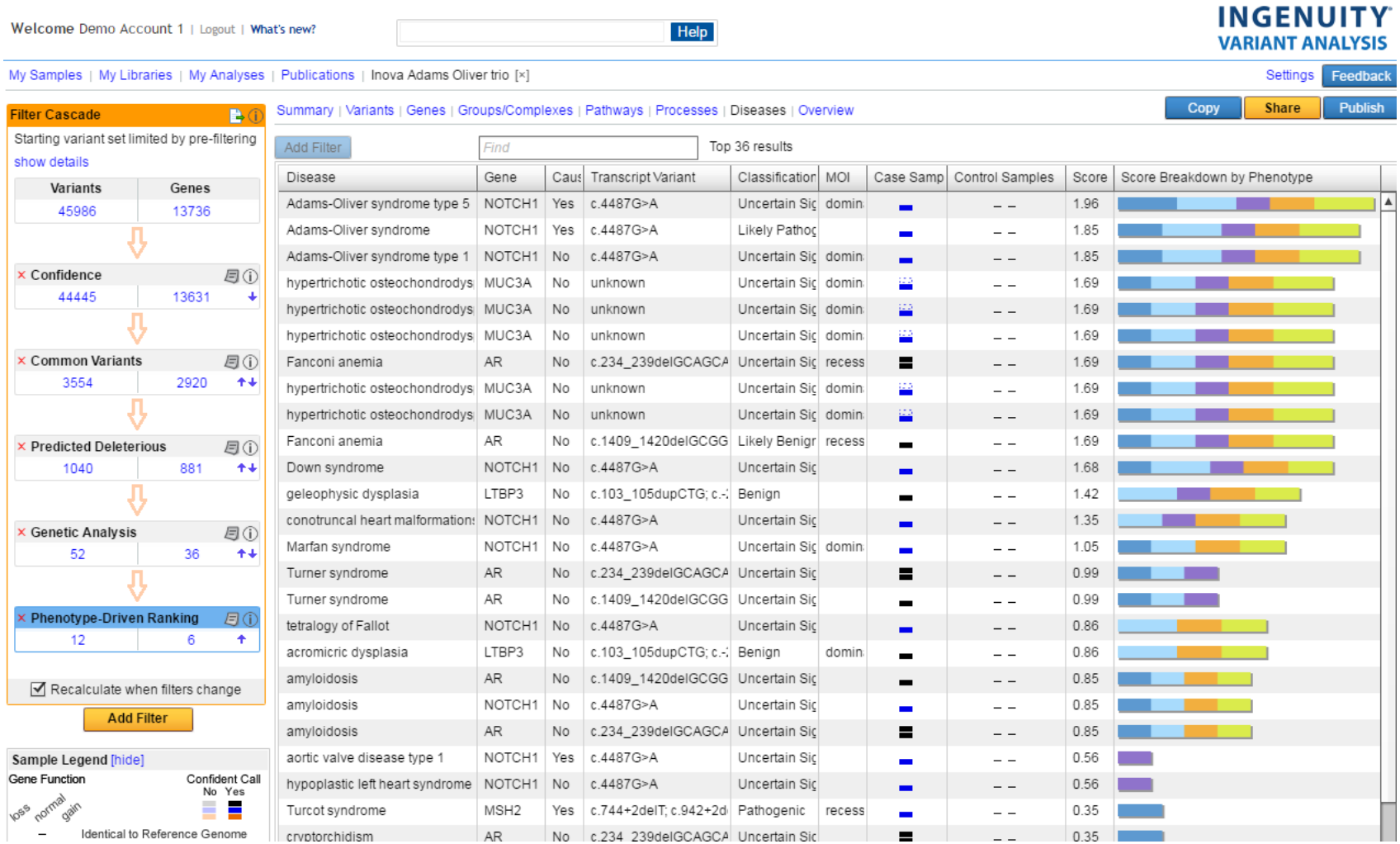
After the genetic analysis filter only variants unique to the proband remain



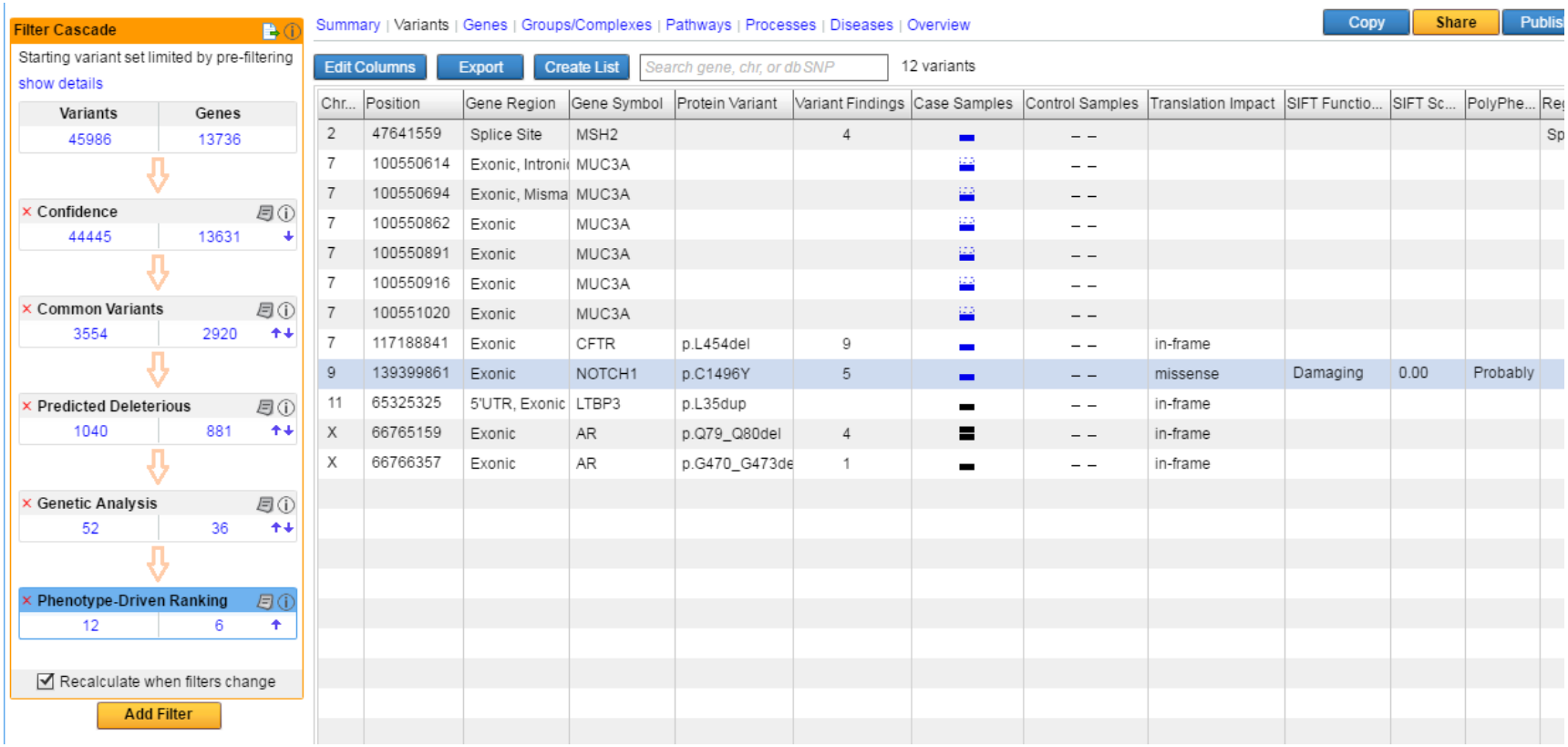
Refine this further using phenotype information…



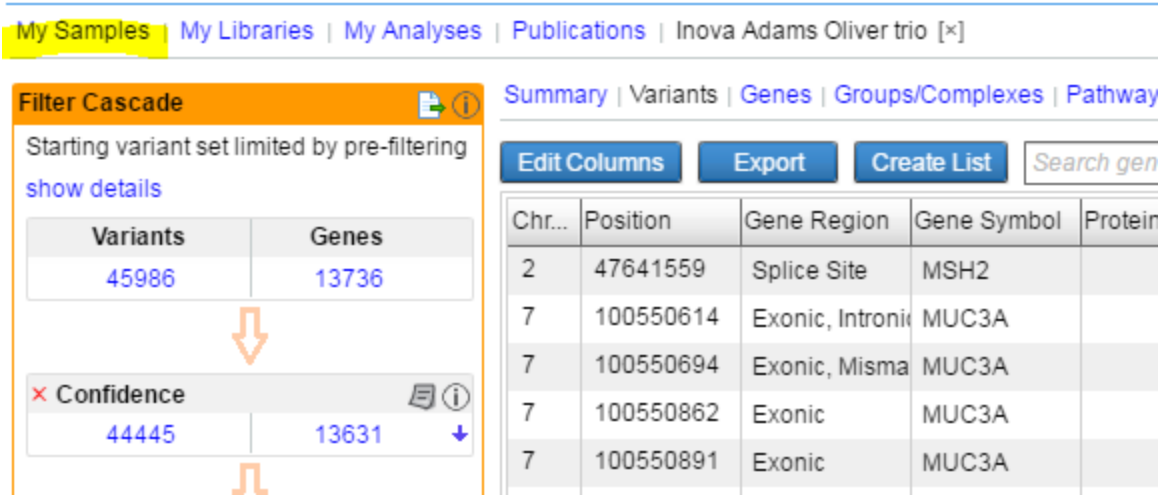
The result is a list of variants prioritized by the relationship with the patients phenotype



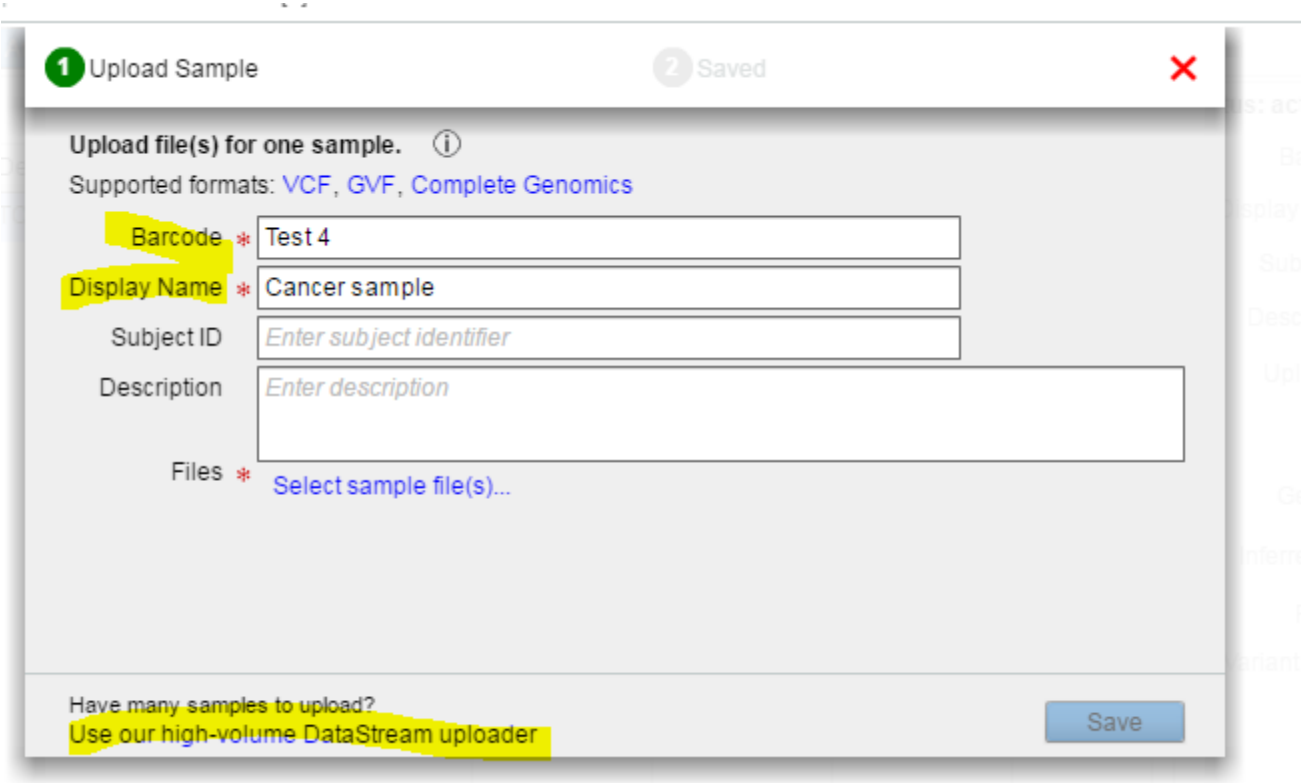
Can view the key genes and variants…



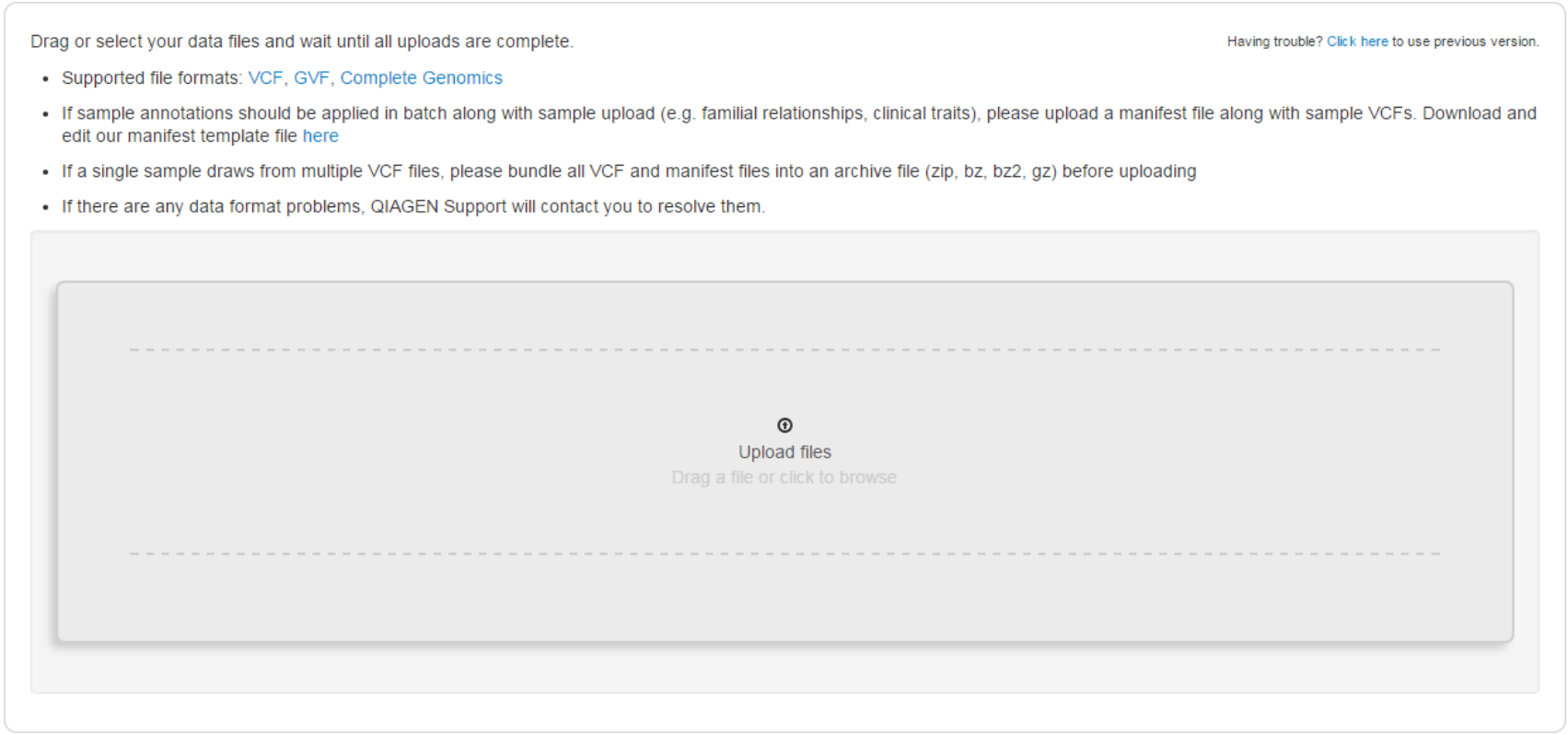
Loading your own data…



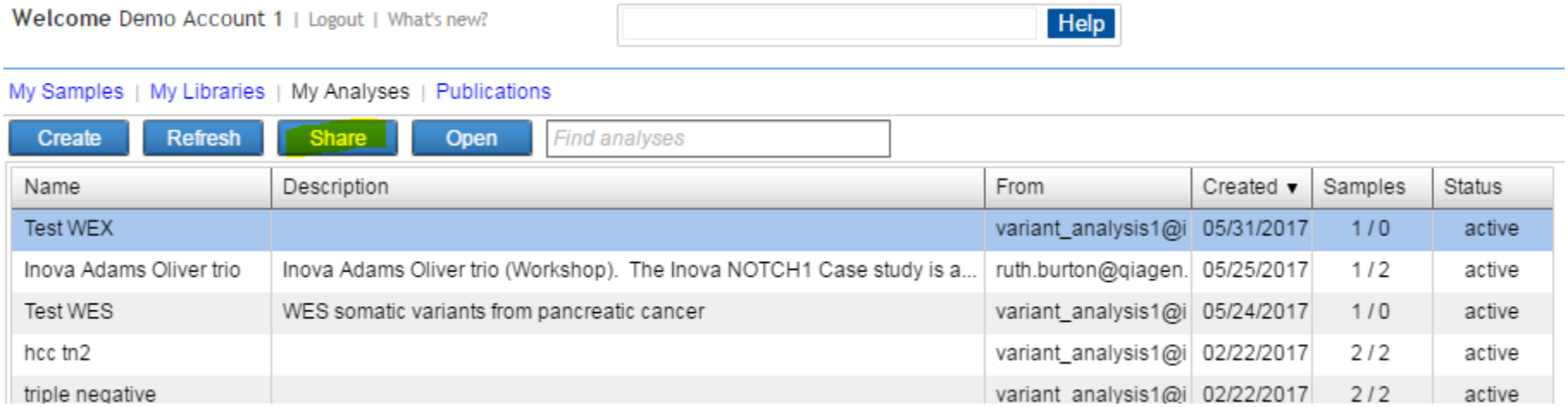
There are two options for large files the datastream is better…



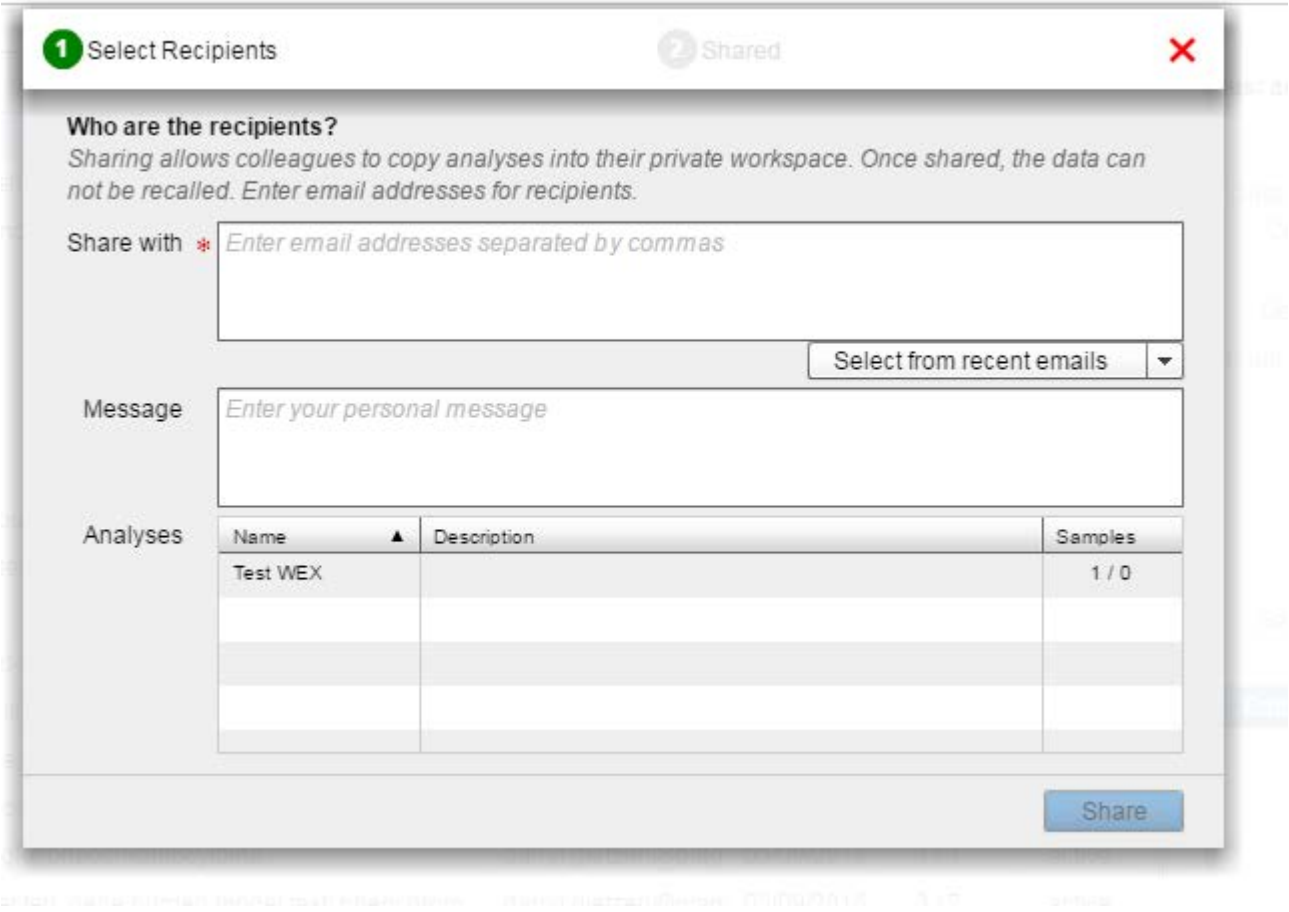
Just drag and drop your files…



Sharing samples, ideal if you want to create your own account



Sharing samples, ideal if you want to create your own account



Any questions?

Come and visit us at our stand
Join us at our presentation on Wednesday

ruth_burton@qiagen.com

www.qiagenbioinformatics.com

Try Ingenuity Variant Analysis today!