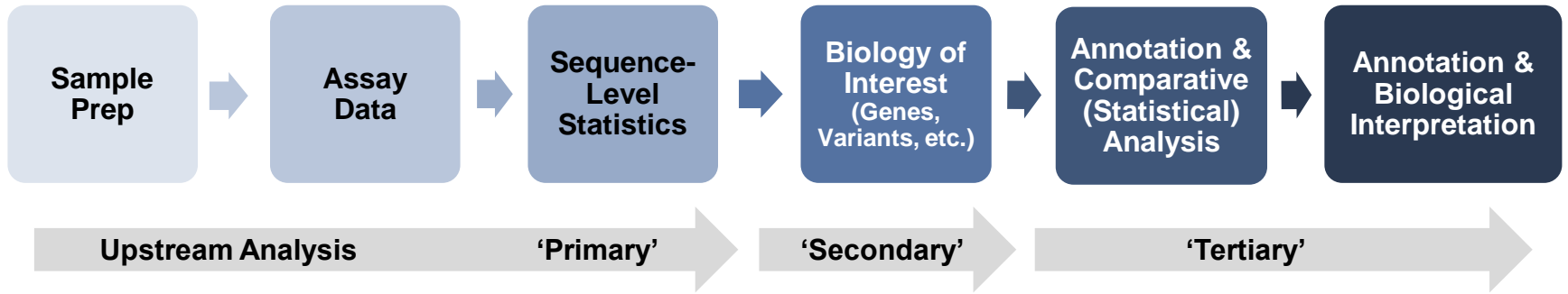




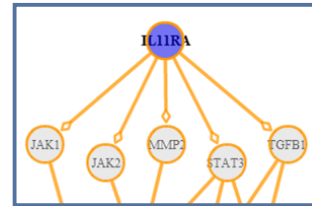
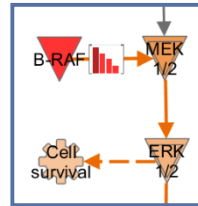
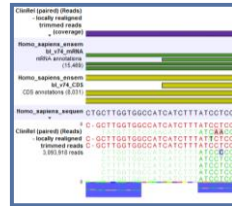
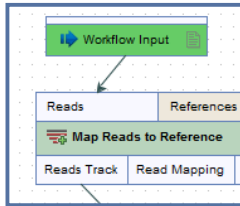
Chr.	Position	Gene	Region	Gene Symbol	Annotations
1	39879851	Exonic, Intron	K3A0754, M4	p.V1305_A1306	in-frame
1	39879859	Exonic, Intron	K3A0754, M4	p.P1308S	in-frame
1	39879877	Exonic, Intron	K3A0754, M4	p.P1314S	missense
1	110121978	Exonic	GN43	p.152_153insS*	in-frame
1	114226255	3'UTR, Exonic	MAG3	p.I1358fs	frameshift
1	179539770	Exonic, Intron	TTN	p.E10236fs, p.E	missense
2	179539770	Exonic, Intron	TTN	p.R2074W, p.R2	missense
2	179640233	Exonic	PPARG	p.L421P, p.L445	missense
3	12475472	Exonic	PPARG		Damaging
3	12475472	Exonic	PPARG		microRNA Bind: MIR216A
3	12475472	Exonic	PPARG		stop gain
3	25639332	3'UTR	RARB		Tolerated
4	90756702	Exonic	SNCA	p.Y39*	missense
4	90756702	Exonic	SNCA	p.E1299D, p.E1	missense
4	112175240	Exonic	APC	p.E684G	Damaging
4	94486725	Exonic	ROR2		missense
4	94486725	Exonic	ROR2		microRNA Bind: MIR300, MIR381
4	94486725	Exonic	ROR2		microRNA Bind: MIR381
10	126677081	3'UTR	CTBP2		1
10	126677081	3'UTR	CTBP2		frameshift
11	32409472	3'UTR	WT1	p.P199fs, p.P48	in-frame
11	32409472	3'UTR	WT1	p.P199fs, p.P48	Damaging
11	64540917	Exonic	SF1	p.P265S	Damaging
11	64540917	Exonic	SF1	p.P265S	missense
12	125397524	Exonic	UBC	p.S115C	frameshift
12	125397524	Exonic	UBC	p.S115C	Damaging
15	48902927	Exonic, ncRNA	SMAD6	p.P47fs	missense
15	48902927	Exonic, ncRNA	SMAD6	p.P47fs	Damaging
15	66995733	Exonic	SREBF1	p.D1124N, p.D1	stop gain
15	66995733	Exonic	SREBF1	p.D1124N, p.D1	missense
17	17716010	Exonic	SREBF1	p.E12*	stop gain
17	17740098	Exonic	SREBF1	p.E12*	stop gain

Finding Causal Variants Using Ingenuity Variant Analysis (IVA)

Dev Mistry, Ph.D.
 Field Applications Scientist
 Devendra.Mistry@qiagen.com



Sample



BRAF c.1798_1799del... p.V600K Likely Pathogenic	BRCA1 c.68_69delAG p.E23fs*17
MYBPC3 c.1624G>C p.E542Q Likely Pathogenic	PIK3CA c.3075C>T p.T1025T Benign

Insight



INGENUITY
PATHWAY ANALYSIS

INGENUITY
VARIANT ANALYSIS



- Introduction
- Data upload and sharing process
- Analysis filter setup
- Results and biological interpretation
- Exporting results
- Summary

What is Ingenuity Variant Analysis and why use it?

Filter Cascade

Variants	Genes
2564011	20349
↓	
× Common Variants	
447848	17520
↓	
× Predicted Deleterious	
7040	5036
↓	
× Genetic Analysis	
28	13
↓	
× Biological Context	
17	5
↓	
× Biological Context	
3	1

Add Filter

Legend [hide]

Function	Call Quality
loss	<20
normal
gain	30+
-	Identical to Reference Genome
[Pattern]	Heterozygous Variant
[Pattern]	Heterozygous/Ambiguous
[Pattern]	Homozygous Variant
[Pattern]	Copy Number Gain/Heterozygous
[Pattern]	Copy Number Gain/Homozygous
[Pattern]	Hemizygous
[Pattern]	Nullizygous
[Pattern]	Gene Fusion
[Pattern]	No genotype

Summary | Variants | Genes | Groups/Complexes | Pathways | Processes | Diseases | Overview

Share

Edit Columns Export Create List Search for gene name/symbol 3 variants

Chr...	Position	Variatio...	Gene Region	Gene Symbol	Protein Variant	Case Samples	Control S
14	65544630	SNP	Exonic, Intronic	MAX	G99D	-- --	---
14	65544703	SNP	Exonic, Intronic	MAX	R66X, R75X	-- --	---
14	65569057	SNP	Exonic	MAX	M1V	-- --	---

Variant: chr14 - 65544703

View: [More Details](#)
[Path to Phenotype](#)
[Variant Findings \(1\)](#)

Gene Symbol: [MAX](#)
 MYC associated factor X

Variant: chr14 | 65544630 | q23.3 | SNP | T

Details | Path to Phenotype | Diseases

Click on lines to see supporting citations.

Findings (12 citations)

[A Myc network accounts for similarities between embryonic stem and cancer cell transcription programs. Cell. \(2010\)](#)

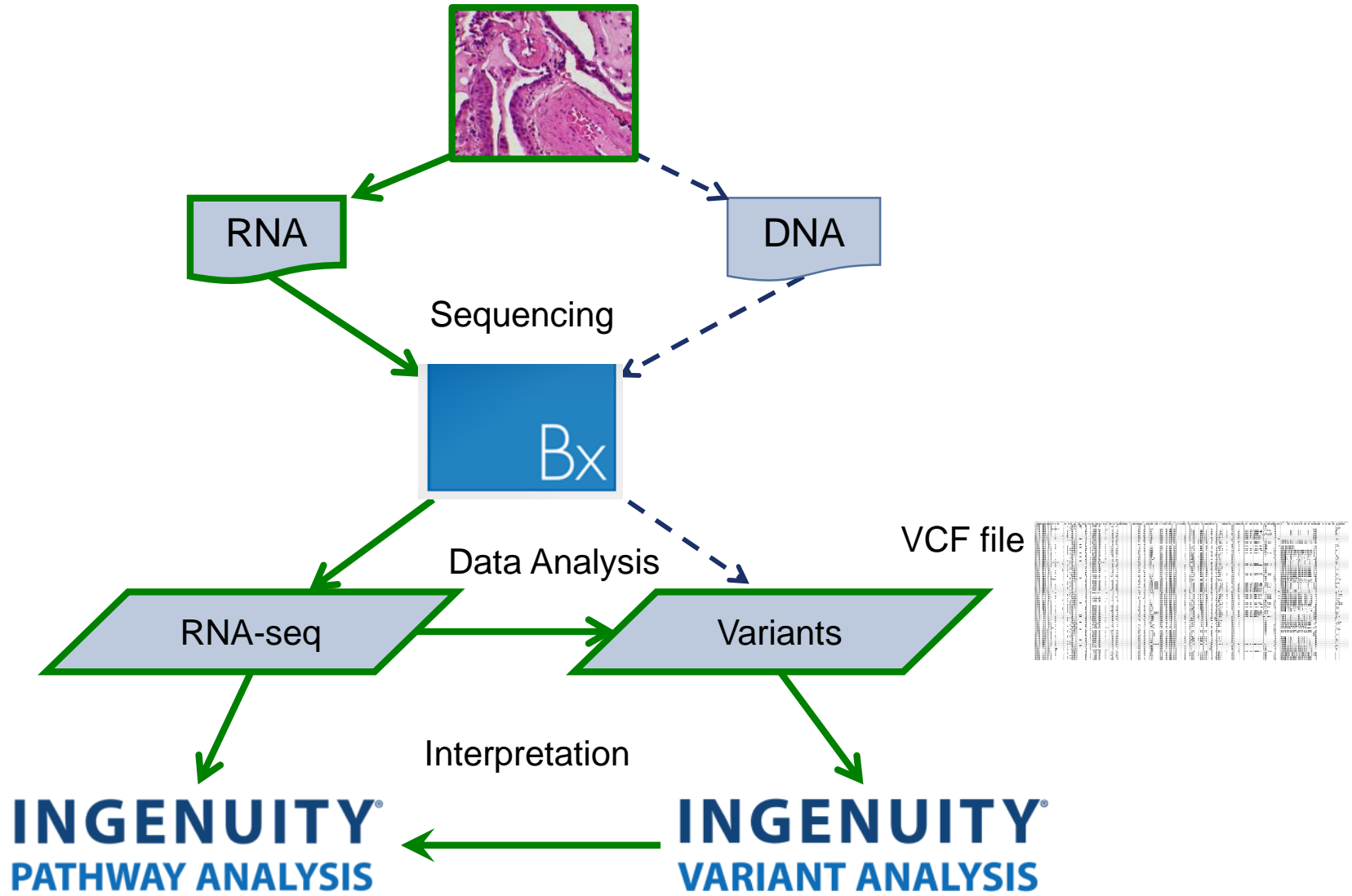
Binding of human **MAX** protein and human **MYCN** protein occurs.

[An atlas of combinatorial transcriptional regulation in mouse and man. Cell. \(2010\)](#)

Binding of mouse **Max** protein and mouse **Mycn** protein occurs.

[The Mvc Transactivation Domain Promotes Global Phosphorylation of the RNA Polymerase II Carboxy-Terminal](#)

Ingenuity Variant Analysis Workflow



Streamlined workflows and a rich toolbox to efficiently process data

Toolbox

Ready-to-Use Workflows

- Preparing Raw Data
- Whole Genome Sequencing
- Whole Exome Sequencing
- Targeted Amplicon Sequencing
 - Annotate Variants (TAS)
 - Filter Somatic Variants (TAS)
 - Identify Known Variants in One Sample (TAS)
 - Identify Somatic Variants from Tumor Normal Pair (TAS)
 - Identify Variants (TAS)
 - Identify and Annotate Variants (TAS)
- Whole Transcriptome Sequencing

Tools

- Genome Browser
- Quality Control
- Preparing Raw Data
- Resequencing Analysis
- Add Information to Variants
- Remove Variants
- Add Information to Genes
- Compare Samples
- Ingenuity Pathway Analysis
- Identify Candidate Variants
- Identify Candidate Genes
- Transcriptomics Analysis
- Helper Tools
- Ingenuity Variant Analysis
- Cloning and Restriction Sites
- Sanger Sequencing
- Epigenomics Analysis
- Workflows

Customize workflows

Workflow Input

QC reports

QC for Target Sequencing (Tue Nov 18 19:19:09 GMT 2014)

Version: CLC Cancer Research Workbench 1.5.2

User: tbonnert

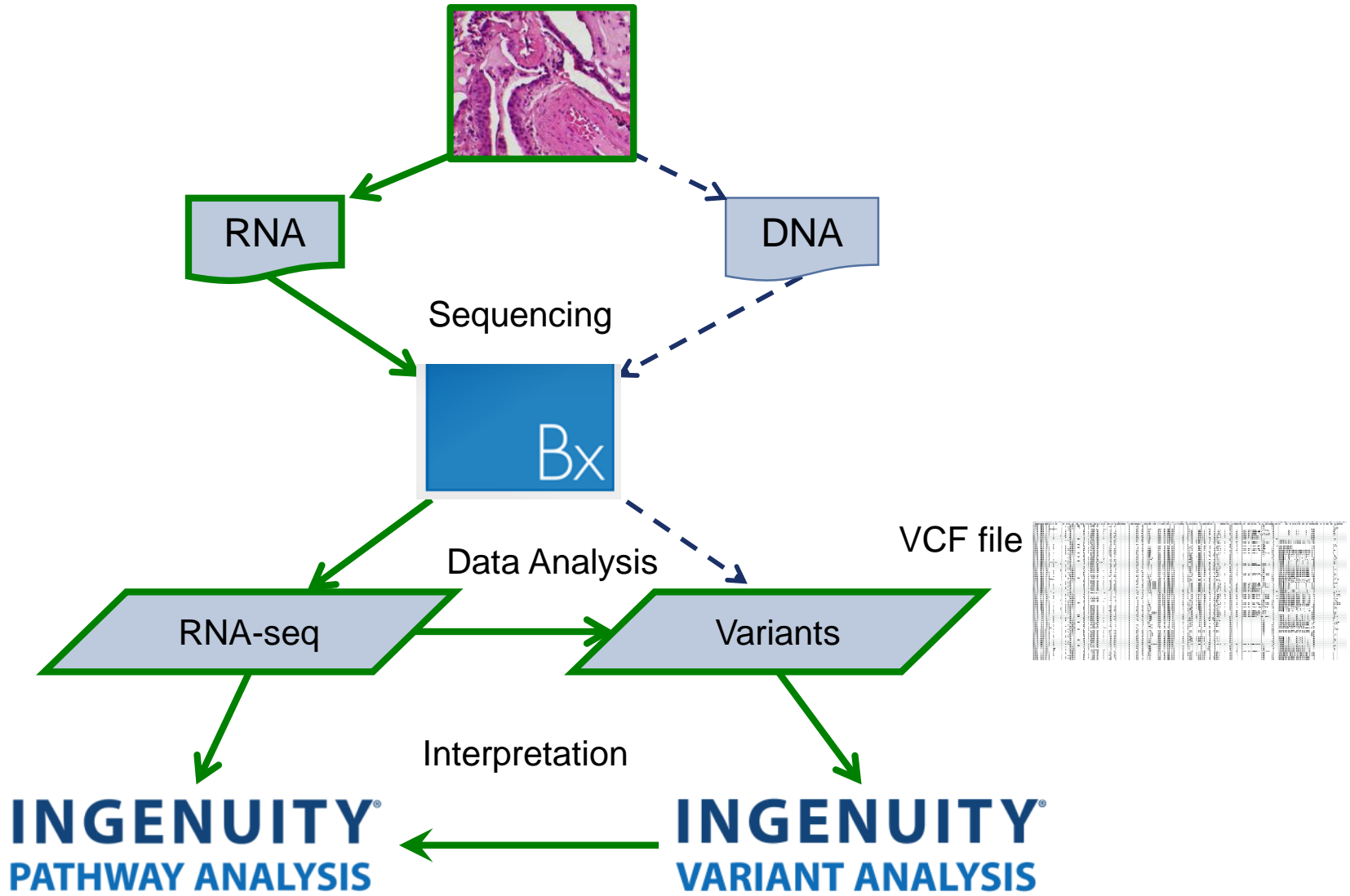
Parameters:

- Track of Target Regions = NGH5-003X_Human_Myeloid_Leukemia
- Report type = 1x, 5x, 10x, 20x, 40x, 80x, 100x
- Minimum coverage = 30
- Ignore non-specific matches = Yes
- Ignore broken pairs = Yes
- Create report = Yes
- Create track = Yes
- Create coverage table = No

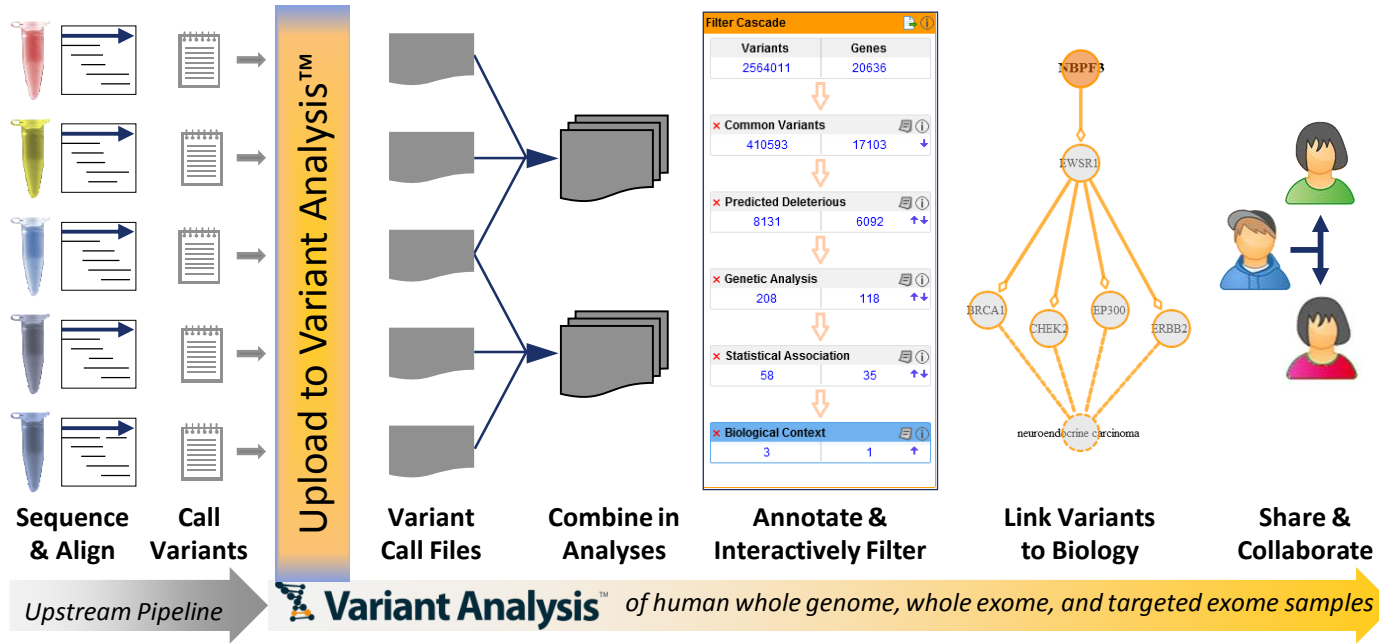
History

Visualization and Validation

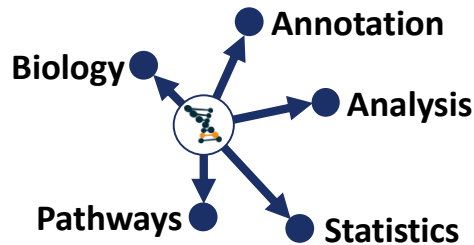
Chromoso...	Region	Type	Reference	Allele	Frequency	Coding region change	Amino acid change	dbSNP
13	32929387	SNV	T	C	99.95	ENST00000544455:c.7397T>C	ENSP00000439902:p.Val2466Ala	169547
17	41246481	SNV	T	C	71.88	ENST00000471181:c.1067A>G	ENSP00000418960:p.Gln356Arg	2889762
17	41258504	SNV	A	C	69.06	ENST00000471181:c.181T>G	ENSP00000418960:p.Cys61Gly	2889762



Ingenuity Variant Analysis



- Stratification Studies ✓
- Large Cancer Studies ✓
- Genetic Disease Cohort ✓
- Trio/Quad Study ✓
- Tumour-Normal Pair ✓
- Personal Genome ✓

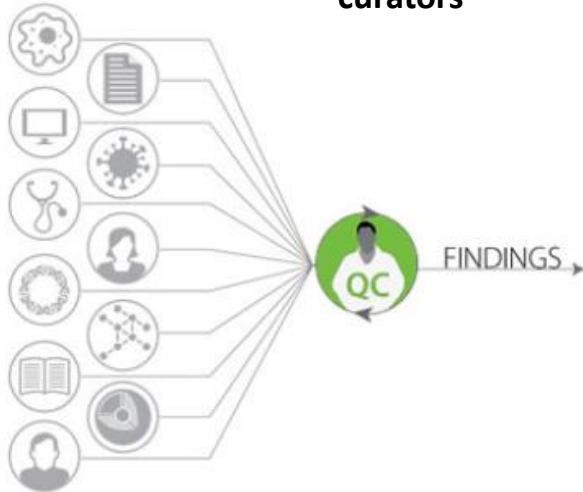


- Pedigree Support
- Disease Identification
- Statistical Burden Testing

Unprecedented Access to Literature Knowledge

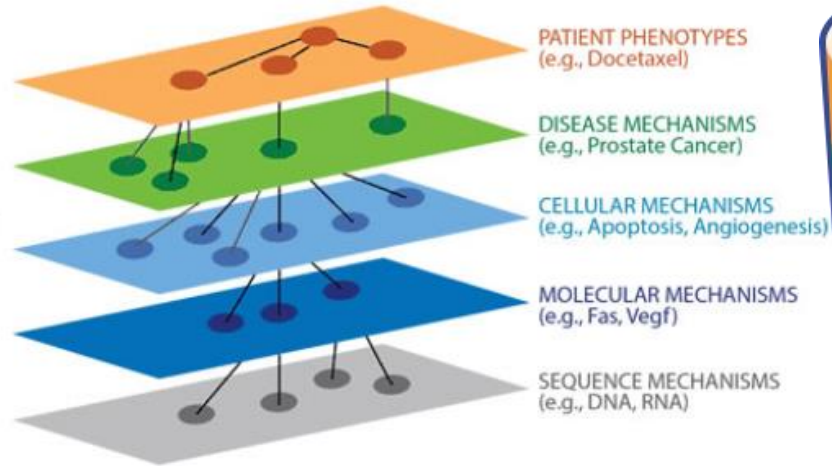
Literature findings

MD/PhD level
curators



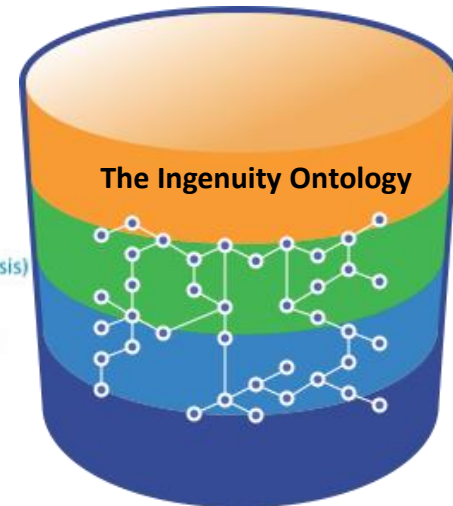
Content Acquisition

Biomedical Ontology

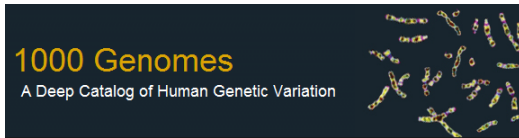


Ingenuity Ontology

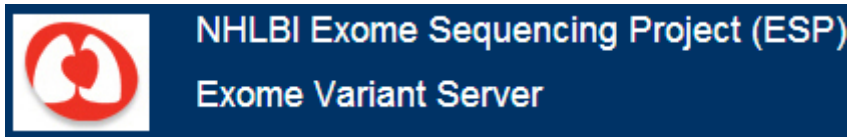
The Ingenuity
Knowledge Base



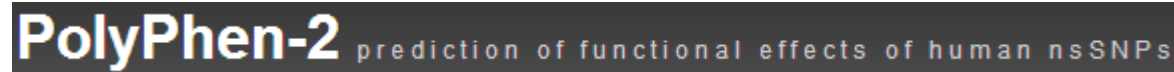
Quality, Context, Coverage, and Timeliness of Content (ca.1/2014)



Mouse Genome Database



Sorting Intolerant From Tolerant



Additionally

- 349,748+ Ph.D./M.D. expert-curated human phenotype-associated mutation findings
- ~3M+ manual literature findings
- 21,458+ curated disease models
- 185,310+ curated pharmacogenetic (PGx) findings

www.allelefrequencycommunity.org

- Leverage the world's largest pool of anonymized allele frequency data
- **Reduces false positives** in analyses by removing variants that are commonly seen in the general population
- Contains Whole Exome AND Whole Genome data
- Better representation of Insertions and Deletions
- **Larger than ANY other public resource**
 - AFC launched with 70,000 samples with >8,000 as whole genomes
 - 12x larger than Exome Variant Server data
- The initial launch version of the database already provides a 43% average false positive rate reduction in a benchmarking set of whole-genome Diagnostic Odyssey cases
- AFC will grow as more people opt-in
 - Launched on 25th February 2015 with 70,000 Samples including 8,000 Whole Genomes
 - Currently at over 100,000 samples, including over 14,000 Whole Genome samples

Use biological associations and molecular interactions

Filter x

Biological Context

Keep only ▼ variants

within 2 hops ▼ upstream ←

that are known or predicted to

Affect ▼

genes listed below or genes implicated in the following diseases, processes, pathways, phenotypes, domains, activities, or biomarker

Enter and select term

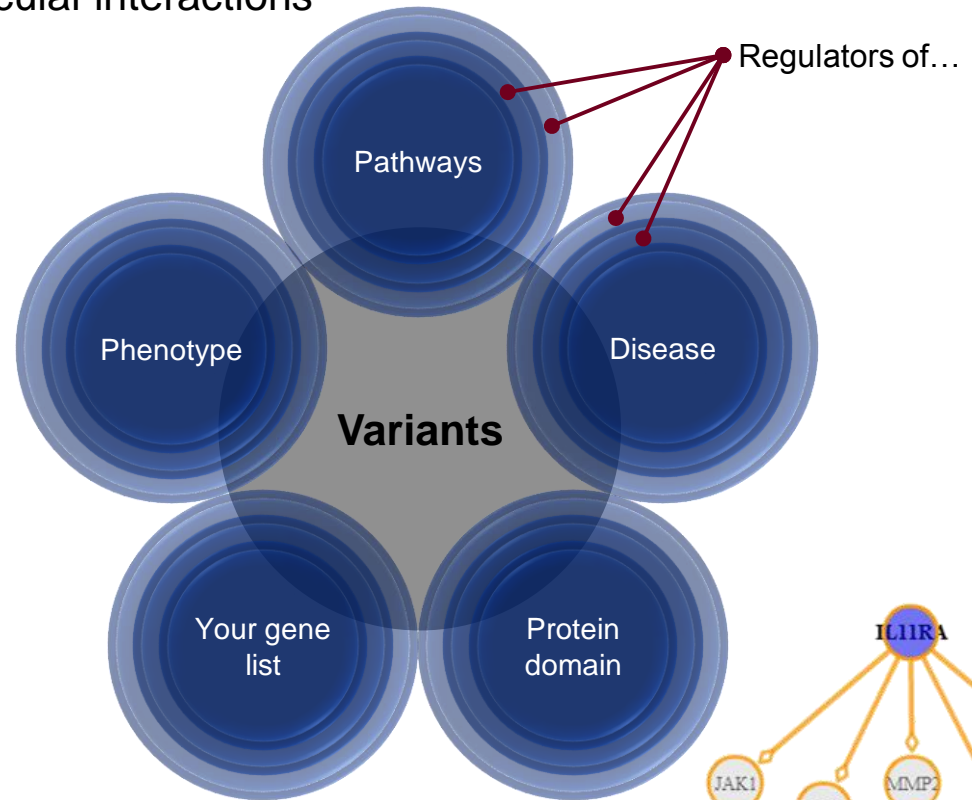
Diseases / Phenotypes / Genes / Signalling pathways / Biological processes / Protein domains / Protein families

[Upload gene list file\(s\)...](#)

and genes within 1 hop ▼ downstream of above

include diseases consistent with the phenotypes above

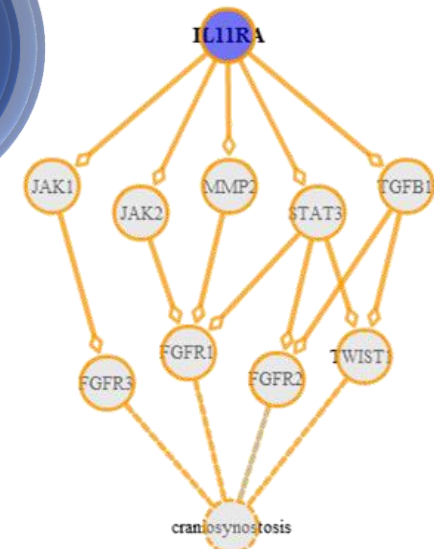
Apply



Findings (55 citations) x

[Rare mutations of FGFR2 causing apert syndrome: identification of the first partial gene deletion, and an Alu element insertion from a new subfamily.](#) *Hum Mutat.* (2009)

- Gain-of-function heterozygous germline mutant human **FGFR2** protein (p.P253R) is observed with autosomal dominant **Apert's syndrome** in human (Study size: multiple individuals).
- Gain-of-function heterozygous germline mutant human **FGFR2** protein (p.S252W) is observed with autosomal dominant **Apert's syndrome** in human (Study size: multiple individuals).



Case Study: Pheochromocytoma (PCC)

Hereditary pheochromocytoma (PCC) is a neuroendocrine tumor of the medulla of the adrenal glands

Whole exome sequencing (Agilent SureSelect) on Illumina Genome Analyzer II

Sequence data obtained from European Nucleotide Archive (ENA)

- <http://www.ebi.ac.uk/ena/data/view/ERR031607-ERR031626>

Published in Nature Genetics (2011); PMID: 2168591

- Exome sequencing identifies MAX mutations as a cause of hereditary pheochromocytoma

Barcode	Display Name	State	Subject	Variants	
ERS025965	924_Hereditary_Pheochromocytoma	case	924	231460	Independent hereditary pheochromocytoma
ERS025967	3037_Hereditary_Pheochromocytoma	case	3037	283434	
ERS025973	3121_Hereditary_Pheochromocytoma	case	3121	243291	
ERS025971	NA11881_Control	control	NA11881	731542	Independent HapMap samples
ERS025969	NA12144_Control	control	NA12144	304008	
ERS025974	NA12892_Control	control	NA12892	681906	
ERS025970	NA12813_Control	control	NA12813	548289	
ERS025966	NA12763_Control	control	NA12763	1098602	
ERS025968	NA12761_Control	control	NA12761	834283	
ERS025972	NA12750_Control	control	NA12750	488217	



Exome sequencing identifies *MAX* mutations as a cause of hereditary pheochromocytoma

Iñaki Comino-Méndez^{1,2,15}, Francisco J Gracia-Aznárez^{2,3,15}, Francesca Schiavi^{4,15}, Iñigo Landa¹, Luis J Leandro-García¹, Rocío Letón¹, Emiliano Honrado⁵, Rocío Ramos-Medina⁶, Daniela Caronia⁷, Guillermo Pita⁷, Álvaro Gómez-Graña¹, Aguirre A de Cubas¹, Lucía Inglada-Pérez^{1,2}, Agnieszka Maliszewska¹, Elisa Taschin⁴, Sara Bobisse⁴, Giuseppe Pica⁸, Paola Loli⁹, Rafael Hernández-Lavado¹⁰, José A Díaz¹¹, Mercedes Gómez-Morales¹², Anna González-Neira⁷, Giovanna Roncador⁶, Cristina Rodríguez-Antona^{1,2}, Javier Benítez^{2,3}, Massimo Mannelli¹³, Giuseppe Opocher^{4,14}, Mercedes Robledo^{1,2} & Alberto Cascón^{1,2}

Examples

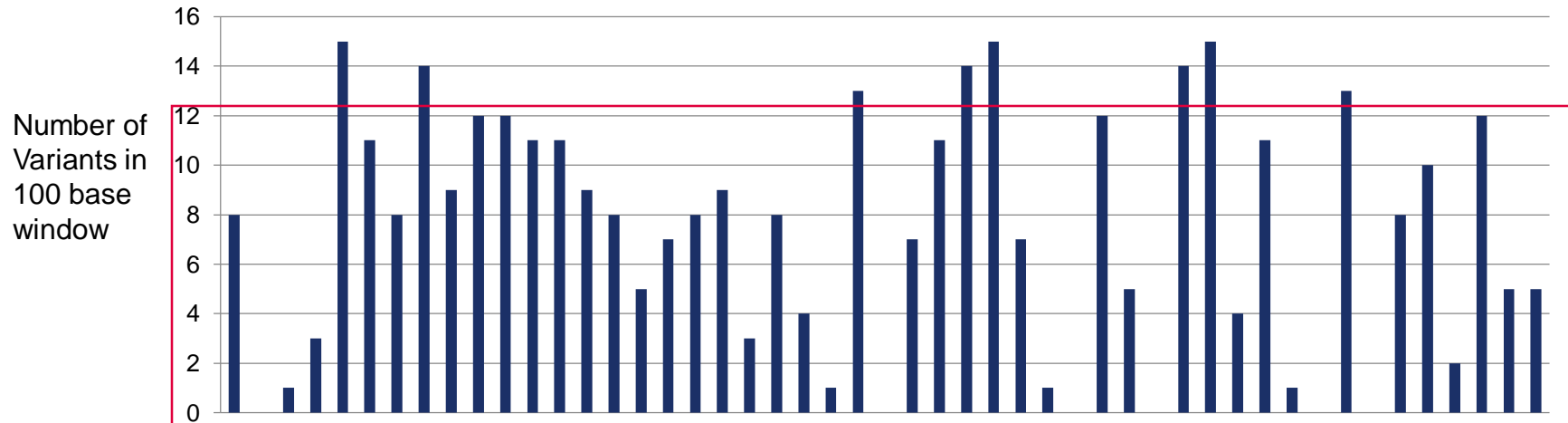
- Tumor vs Normal
- Trio (Hereditary)
- Stratification (affected vs unaffected)

Live Demo

SLIDE EXPLAINING NOISE FILTER



SLIDE EXPLAINING NOISE FILTER



Keep regions not in the top $x\%$

Will ignore top $x\%$ most exonicly variable 100 base regions

Keep outside top x% most exonically variable genes

Identification and Removal of Artifacts

Counts based on single sample, qual 20, allele frequency < 3%

	A	B
1	Gene Symbol	Count
2	MUC4	129
3	PTPRN2	98
4	MUC6	95
5	HLA-DRB1	89
6	MUC16	80
7	PRSS1/PRSS3	63
8	CSF2RA	60
9	PDE4DIP	53
10	BAGE3; BAGE4; BAGE2	53
11	PRIM2	48
12	MAP2K3	46
13	KCNJ12; KCNJ18	45
14	C9orf96	44
15	CDK11A/CDK11B	43
16	MUC12	40
17	AQP7	40
18	TUBGCP2	39
19	CDH4	39
20	HLA-DRB5	39
21	SLC22A2	39
22	C7orf50	36
23	ANKRD36B (includes others)	34
24	PRKAG2	32
25	TTC34	31
26	OR8U1	31
27	EPHA8	29
28	FRG2 (includes others)	29
29	SORCS2	29
30	COL5A1	29

I	J	K	L	M	N	O
Variants per Gene	Count of Genes	Pct of Genes in Analysis	Cumulative Pct of Genes		Cumulative Sum of Variants	Cumulative Pct of Variants
1	3795	56.68%	56.68%		3795	23.40%
2	1307	19.52%	76.21%		6409	39.51%
3	602	8.99%	85.20%		8215	50.64%
4	322	4.81%	90.01%		9503	58.58%
5	194	2.90%	92.91%		10473	64.56%
6	114	1.70%	94.61%		11157	68.78%
7	88	1.31%	95.92%		11773	72.58%
8	48	0.72%	96.64%		12157	74.95%
9	32	0.48%	97.12%		12445	76.72%
10	28	0.42%	97.54%		12725	78.45%
11	32	0.48%	98.01%		13077	80.62%
12	16	0.24%	98.25%		13269	81.80%
13	13	0.19%	98.45%		13438	82.84%
14	15	0.22%	98.67%		13648	84.14%
15	13	0.19%	98.86%		13843	85.34%
16	11	0.16%	99.03%		14019	86.43%
17	7	0.10%	99.13%		14138	87.16%
18	5	0.07%	99.21%		14228	87.71%
19	3	0.04%	99.25%		14285	88.06%
20	6	0.09%	99.34%		14405	88.80%
21	1	0.01%	99.36%		14426	88.93%
22	2	0.03%	99.39%		14470	89.21%
23	2	0.03%	99.42%		14516	89.49%
24	7	0.10%	99.52%		14684	90.52%

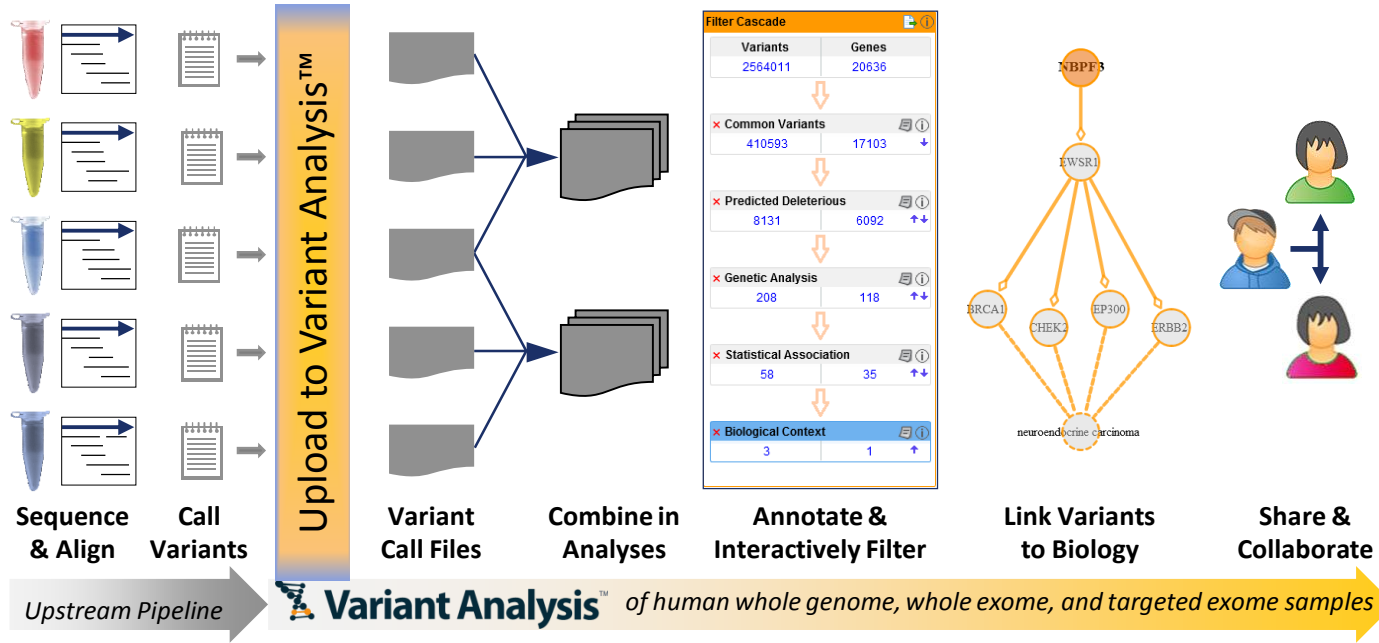
For example, if set at 5%:

Keep genes that are not in the top 5% most exonically variable

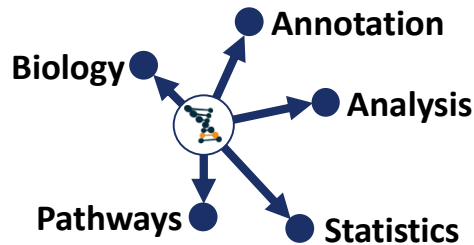
Will ignore the top 5% most exonically variable genes

Summary

Ingenuity Variant Analysis



- Stratification Studies ✓
- Large Cancer Studies ✓
- Genetic Disease Cohort ✓
- Trio/Quad Study ✓
- Tumour-Normal Pair ✓
- Personal Genome ✓



- Pedigree Support
- Disease Identification
- Statistical Burden Testing

My Samples | My Analyses | Publications | Claudin ProbVar [x]

Summary | Variants | Genes

Filter Cascade

Variants	Genes
46076	9648
↓	
× Confidence	
46076	9648
↓	
× Common Variants	
16809	5996
↓	
× Predicted Deleterious	
5583	3474
↓	
× Genetic Analysis	
38	5
↓	
× Biological Context	
38	5

Recalculate when filters change

Add Filter

Chr...	Position	Gene
6	31236668	3'UT
6	31236767	3'UT
6	31236800	3'UT
6	31236821	3'UT
6	31236853	3'UT
6	31236862	3'UT
6	31237773	Exon
6	31238027	Exon
6	31238909	Exon
6	31238931	Exon
6	31238995	Exon
6	31239050	Exon
6	31239100	Exonic
6	31239101	Exonic
6	31239501	Exonic
8	11700213	3'UTR
8	11700373	3'UTR
8	11700676	3'UTR

Filter

Confidence **Rename**

Keep only variants which satisfy all of these criteria:

- Call quality is at least in any case or at least in any control
- AND**
- Variant passed upstream pipeline filtering
- AND**
- Read depth is at least in any sample
- AND**
- Allele fraction is at least % in any sample
- AND**
- Outside top % most exonically variable 100base windows in healthy public genomes
- AND**
- Outside top % **most exonically variable genes** in healthy public genomes (1000 Genomes)

Subsequent filters only treat a variant as present for samples that also satisfy the Keep criteria.

Apply

Filter Cascade

Variants	Genes
46076	9648
↓	
× Confidence	
46076	9648
↓	
× Common Variants	
16809	5996
↓	
× Predicted Deleterious	
5583	3474
↓	
× Genetic Analysis	
38	5
↓	
× Biological Context	
38	5

Recalculate when filters change

Filter Rename

Common Variants

variants that are observed in any of these populations with an allele frequency of

- at least 3 % in the 1000 Genomes Project
- at least 3 % in the ExAC
- at least 3 % of all NHLBI ESP exomes
- at least 3 % in the Allele Frequency Community (includes ExAC and CGI)

OR

are present in dbSNP

* The public Complete Genomics genomes are included in the AFC

My Samples | My Analyses | Publications | Claudin ProbVar [x] Feedback

Summary | Variants | Genes | Groups/Complexes | Pathways | Processes | Diseases | Overview Share Publish

Filter Cascade

Variants	Genes
46076	9648
↓	
× Confidence	
46076	9648
↓	
× Common Variants	
16809	5996
↓	
× Predicted Deleterious	
5583	3474
↓	
× Genetic Analysis	
38	5
↓	
× Biological Context	
38	5

Recalculate when filters change

Add Filter

Chr...	Position	Gene	Impact	SIFT Fu
6	31236668	3'UTR		
6	31236767	3'UTR		
6	31236800	3'UTR		
6	31236821	3'UTR		
6	31236853	3'UTR		
6	31236862	3'UTR		
6	31237773	Exon		
6	31238027	Exon		
6	31238909	Exon		
6	31238931	Exon		
6	31238995	Exon		
6	31239050	Exon		
6	31239100	Exon		
6	31239101	Exon		
6	31239501	Exon		
8	11700213	3'UTR		
8	11700373	3'UTR		
8	11700676	3'UTR		

Predicted Deleterious

Keep only variants that are experimentally observed to be associated with a phenotype:

Disease-associated according to computed ACMG Guidelines classification

- Pathogenic
- Likely Pathogenic
- Uncertain Significance
- Likely Benign
- Benign
- Listed in HGMD®

OR

are associated with gain of function of a gene

- Established in the Literature
- Gene Fusion
- Inferred activating mutation by Ingenuity
- Predicted gain of function by BSIFT
- microRNA Binding Site
- Copy Number Gain

OR

are associated with loss of function of a gene

- Frameshift, in-frame indel, or start/stop codon change
- Missense unless predicted tolerated by SIFT or PolyPhen-2
- Nullizygous
- Splice site loss up to bases into intron or as predicted by MaxEntScan
- Deleterious to a microRNA

Apply

Legend [show]

My Samples | My Analyses | Publications | Claudin ProbVar [x]

Summary | Variants | Genes | Groups/Complexes | Pathways | Processes | Diseases | Overview

Share Publish

Filter Cascade

Variants	Genes
46076	9648
↓	
× Confidence	
46076	9648
↓	
× Common Variants	
16809	5996
↓	
× Predicted Deleterious	
5583	3474
↓	
× Genetic Analysis	
38	5
↓	
× Biological Context	
38	5

Recalculate when filters change

Add Filter

Filter

Biological Context **Rename**

Keep only variants

within 1 hop upstream

that are known or predicted to

Affect

genes listed below or genes implicated in the following diseases, processes, pathways, phenotypes, domains, activities, or biomarkers

Enter and select term

× epithelial-mesenchymal transition [process]

Genes

3,3'-diindolylmethane

ABL1

AGT

AKT1

AKT2

AKT3

ALX1

AMELX

ARHGAP21

beta-estradiol

BMI1

BMP2

BMP7

bosutinib

C1orf61

CAV1

CD44

CDC42

CDH1

CDH11

CLIC4

[Upload gene list file\(s\)...](#)

and genes within 1 hop downstream of above

include diseases consistent with the phenotypes above

Apply

My Samples | My Analyses | Publications | Claudin ProbVar [x]

Summary | Variants

Filter Cascade

Variants	Genes
46076	9648
↓	
Confidence	
44561	9564
↓	
Common Variants	
8020	3152
↓	
Predicted Deleterious	
532	505
↓	
Genetic Analysis	
16	11
↓	
Biological Context	
5	2

Recalculate when filters change

Add Filter

Biological Context

Cancer

Common Variants

Confidence

Custom

Genetic Analysis

Pharm

Physi

Predict

Statist

User-I

Filter

Cancer Driver Variants **Rename**

Keep only [v] variants that are found in

- Cancer-associated mouse knockout phenotypes
[View list of phenotypes](#)
- Cancer-associated cellular processes with [appropriate directionality] [v]
[View list of processes](#)
- Cancer-associated pathways with [appropriate directionality] [v]
[View list of pathways](#)
- Cancer therapeutic targets
[View list of drug targets](#)
- Published cancer literature [variant level] [v] findings
- Known or predicted cancer subnetwork regulatory sites
[View list of disease genes](#)
- COSMIC at a frequency [greater than or equal to] [v] [0.1] [v] %
- TCGA at a frequency [greater than or equal to] [v] [0.1] [v] %

AND

Involved in any of the diseases listed below

[v]

- Breast Cancer

Apply

Filter Cascade

Variants	Genes
46076	9648
↓	
Confidence	
44561	9564
↓	
Common Variants	
8020	3152
↓	
Predicted Deleterious	
532	505
↓	
Genetic Analysis	
16	11
↓	
Biological Context	
5	2

Recalculate when filters change

Add Filter

Chr...	Position	Gene Region	Gene Symbol	Protein Variant	Case Samples	Control Sample
11	534288	Exonic	HRAS	p.G12D	-- -- --	-- -- --
17	7577099	Exonic	TP53	p.R121K, p.R148	-- -- --	-- -- --
17	7577534	Exonic	TP53	p.R117S, p.R210	-- -- --	-- -- --
17	7578461	5'UTR, Exonic	TP53	p.V118F, p.V157	-- -- --	-- -- --
17	7578526	5'UTR, Exonic	TP53	p.C135F, p.C3F	-- -- --	-- -- --

Legend [hide]

Function

loss normal gain

Confident Call

No Yes

- Identical to Reference Genome
- Heterozygous Variant
- Heterozygous/Ambiguous
- Homozygous Variant
- Copy Number Gain/Heterozygous
- Copy Number Gain/Homozygous
- Hemizygous
- Nullizygous
- Gene Fusion
- No genotype

Variant: chr11 | 534288 | SNV

View: [More Details](#)
[Path to Phenotype](#)
[Variant Findings \(60\)](#)
[Pharmacogenetics \(375\)](#)

Gene Symbol: **HRAS**
 Harvey rat sarcoma viral oncogene homolog

dbSNP ID: [104894230](#)
 Cytoband: p15.5
 COSMIC ID: [484](#) [Validate]
 99915

Position: [chr11:534288](#) [IGV] [UCS]
 HGMD: [CM081305](#)

Classification: [Uncertain Significance](#)

Gene Region: Exonic
 Protein Variant: p.G12D
 Transcript Variant: c.35G>A
 Translation Impact: missense
 SIFT Function Prediction: Damaging
 PolyPhen-2 Function: Benign

Filter Cascade

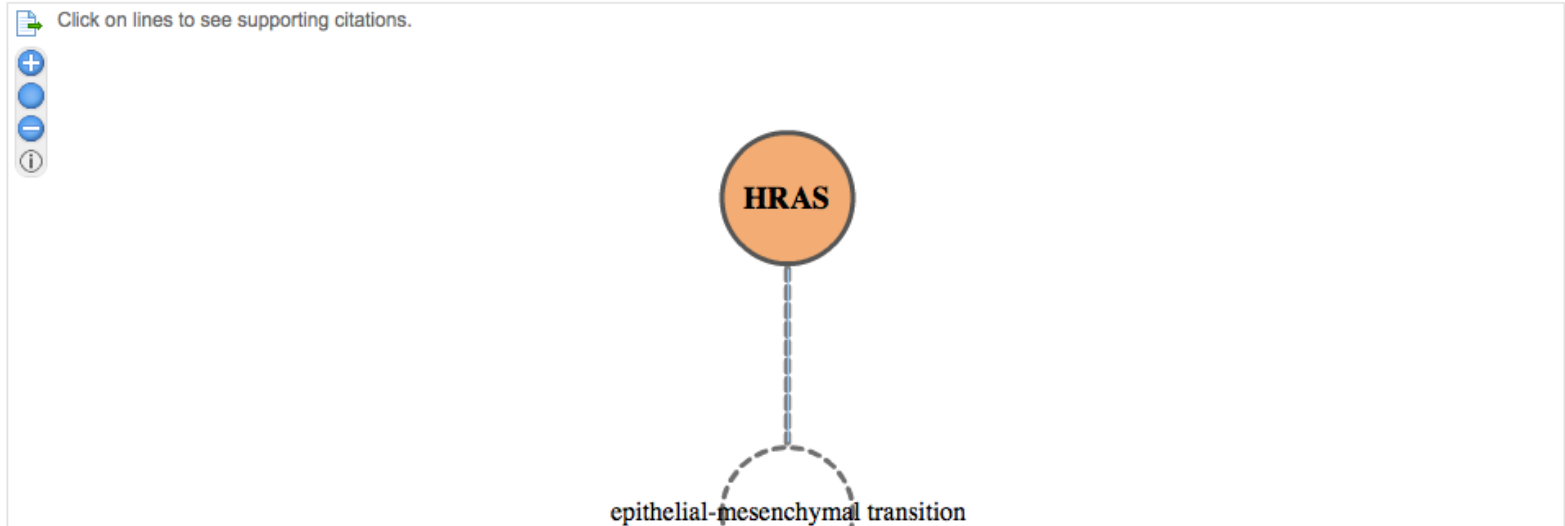
Variants	Genes
46076	9648
↓	
Confidence	
44561	9564

Chr...	Position	Gene Region	Gene Symbol	Protein Variant	Case Samples	Control Sample
11	534288	Exonic	HRAS	p.G12D	-- -- --	-----
17	7577099	Exonic	TP53	p.R121K, p.R148G	-----	-----
17	7577534	Exonic	TP53	p.R117S, p.R216C	-----	-----

Variant: chr11 | 534288 | SNV

View: [More Details](#)
[Path to Phenotype](#)
[Variant Findings \(60\)](#)
[Pharmacogenetics \(375\)](#)

Variant: chr11 | 534288 | p15.5 | SNV | T



Symbol: **HRAS**
 Harvey rat sarcoma viral oncogene homolog

NP ID: 104894230
 band: p15.5
 MIC ID: 484 [Validate]
 99915

Position: chr11:534288 [IGV] [UCS]
 HGMD: CM081305

Classification: **Uncertain Significance**

Gene Region: Exonic
 Protein Variant: p.G12D
 Nucleotide Variant: c.35G>A

Translation: missense
 Impact

Function Prediction: Damaging
 PolyPhen-2
 Function: Benign

Findings (1 citations)

Expression of activated M-Ras in a murine mammary epithelial cell line induces epithelial-mesenchymal transition and tumorigenesis. *Oncogene*. (2004)

- Activated mutant **HRAS** protein (p.G12V) increases epithelial mesenchymal transition of SCp2 cells.

Filter Cascade

Variants	Genes
46076	9648
↓	
Confidence	
44561	9564

Chr...	Position	Gene Region	Gene Symbol	Protein Variant	Case Samples	Control Sample
11	534288	Exonic	HRAS	p.G12D	-- -- --	-----
17	7577099	Exonic	TP53	p.R121K, p.R148G	-----	-----
17	7577534	Exonic	TP53	p.R117S, p.R216C	-----	-----

Variant: chr11 | 534288 | SNV

- View: [More Details](#)
- [Path to Phenotype](#)
- [Variant Findings \(60\)](#)
- [Pharmacogenetics \(375\)](#)

Variant: chr11 | 534288 | p15.5 | SNV | T

Findings (40 citations)

[The Exomes of the NCI-60 Panel: A Genomic Resource for Cancer Biology and Systems Pharmacology. *Cancer Res.* \(2013\)](#)

- Somatic missense heterozygous mutant human **HRAS** gene (c.35G>A translating to p.G12D) is associated with carcinoma in human breast (observed in 1 of 1 samples).
- Somatic missense heterozygous mutant human **HRAS** gene (c.35G>A translating to p.G12D) is associated with carcinoma in human breast (observed in 1 of 1 samples).

[Frequent Mutation of the PI3K Pathway in Head and Neck Cancer Defines Predictive Biomarkers. *Cancer Discov.* \(2013\)](#)

- Somatic missense mutant human **HRAS** gene (c.35G>A translating to p.G12D) is associated with squamous-cell carcinoma in human head and neck (observed in 2 of 7 samples).
- Somatic missense mutant human **HRAS** gene (c.35G>A translating to p.G12D) is associated with squamous-cell carcinoma in human head and neck (observed in 2 of 7 samples).

[RAS Mutations Are Associated With the Development of Cutaneous Squamous Cell Tumors in Patients Treated With RAF Inhibitors. *J Clin Oncol.* \(2012\)](#)

- Somatic missense mutant human **HRAS** gene (c.35G>A translating to p.G12D) is associated with keratoacanthoma in skin from human chest (observed in 1 of 1 samples).

[RAS mutations in cutaneous squamous-cell carcinomas in patients treated with BRAF inhibitors. *N Engl J Med.* \(2012\)](#)

- Somatic missense mutant human **HRAS** gene (c.35G>A translating to p.G12D) is associated with keratoacanthoma in skin from human leg (observed in 1 of 4 samples).
- Somatic missense mutant human **HRAS** gene (c.35G>A translating to p.G12D) is associated with keratoacanthoma in skin from human torso (observed in 1 of 7 samples).

[Postzygotic HRAS and KRAS mutations cause nevus sebaceous and Schimmelpenning syndrome. *Nat Genet.* \(2012\)](#)

- Mutant human **HRAS** gene (c.35G>A) is associated with nevus sebaceous in human.

[HRAS mutants identified in Costello syndrome patients can induce cellular senescence: possible implications for the pathogenesis of Costello syndrome. *J Hum Genet.* \(2011\)](#)

- Change of function heterozygous germline mutant human **HRAS** protein (p.G12D, alternately c.35G>A) is observed with childhood-onset Costello syndrome in human (unknown geographic location).

[Frequent mutations of chromatin remodeling genes in transitional cell carcinoma of the bladder. *Nat Genet.* \(2011\)](#)

- Somatic missense mutant human **HRAS** gene (c.35G>A translating to p.G12D) is associated with carcinoma in human urinary bladder (observed in 2 of 11 samples).
- Somatic missense mutant human **HRAS** gene (c.35G>A translating to p.G12D) is associated with carcinoma in human urinary bladder (observed in 2 of 11 samples).

Symbol: **HRAS**
Harvey rat sarcoma viral oncogene homolog

dbSNP ID: [104894230](#)

Cytoband: p15.5

SMIC ID: [484](#) [Validate]
99915

Position: chr11:534288 [IGV] [UCS]
HGMD: [CM081305](#)

Classification: [Uncertain Significance](#)

Gene Region: Exonic

Protein Variant: p.G12D

Genotype Variant: c.35G>A

Translation Impact: missense

Protein Function Prediction: Damaging

PolyPhen-2 Function: Benign

Filter Cascade

Variants	Genes
46076	9648

↓

Confidence

44561	9564
-------	------

↓

Common Variants

8020	3152
------	------

↓

Predicted Deleterious

532	505
-----	-----

↓

Genetic Analysis

Chr...	Position	Gene Region	Gene Symbol	Protein Variant	Case Samples	Control Sample
11	534288	Exonic	HRAS	p.G12D	-- -- --	-----
17	7577099	Exonic	TP53	p.R121K, p.R148	-----	-----
17	7577534	Exonic	TP53	p.R117S, p.R210	-----	-----
17	7578461	5'UTR, Exonic	TP53	p.V118F, p.V157	-----	-----
17	7578526	5'UTR, Exonic	TP53	p.C135F, p.C3F	-----	-----

Variant: chr11 | 534288 | SNV

View : [More Details](#)
[Path to Phenotype](#)
[Variant Findings \(60\)](#)
[Pharmacogenetics \(375\)](#)

Gene Symbol : **HRAS**
 Harvey rat sarcoma viral oncogene homolog

dbSNP ID : [104894230](#)
 Cytoband : p15.5
 COSMIC ID : [484](#) [Validate]
 99915

Position : [chr11:534288](#) [IGV] [UCS]
HGMD : CM081305

Classification : [Uncertain Significance](#)

HGMD® Professional 2014.1



HGMD accession	Disease/phenotype	Gene symbol	Codon change	Amino acid change	Codon number	Feedback
CM081305	Costello syndrome	HRAS	GGC-GAC	Gly-Asp	12	Feedback

The G12D substitution exhibits a shift in polarity from non-polar to negatively charged and displays a decrease in Kyte-Doolittle hydrophobicity from -0.4 to -3.5. Approximately 1.95% of missense mutations in HGMD are Gly-Asp. The mutation occurs 178 amino acids from the end of the protein.

Literature citation	Citation type	Notes
1. Lo (2008) <i>J Med Genet</i> 45 : 167 PubMed: 18039947	Primary literature report	None
2. Niihori (2011) <i>J Hum Genet</i> 56 : 707 PubMed: 21850009	Functional characterisation	None
3. Cheng (2012) <i>PLoS Comput Biol</i> 8 : e1002738 PubMed: 23093928	Functional characterisation	Systemic impact quantified as a combination of protein stability change and pathway perturbation.

Extra information

Coding strand genomic sequence (GRCh37.3) CGGAATATAAGCTGCTGGTGGCGGGCG(G/A)CGGTCTGGCAAGAGTGGCTGACCATCCA

Filter Cascade

Variants	Genes
46076	9648
↓	
Confidence	
44561	9564
↓	
Common Variants	
8020	3152
↓	
Predicted Deleterious	
532	505
↓	
Genetic Analysis	
16	11
↓	
Biological Context	
5	2

Recalculate when filters change

Add Filter

Chr...	Position	Gene Region	Gene Symbol	Protein Variant	Case Samples	Control Sample
11	534288	Exonic	HRAS	p.G12D	- - - - -	- - - - -
17	7577099	Exonic	TP53	p.R121K, p.R148G	- - - - -	- - - - -
17	7577534	Exonic	TP53	p.R117S, p.R210C	- - - - -	- - - - -
17	7578461	5'UTR, Exonic	TP53	p.V118F, p.V157L	- - - - -	- - - - -
17	7578526	5'UTR, Exonic	TP53	p.C135F, p.C3F	- - - - -	- - - - -

Variant: chr11 | 534288 | SNV

View : [More Details](#)
[Path to Phenotype](#)
[Variant Findings \(60\)](#)
[Pharmacogenetics \(375\)](#)

Gene Symbol : **HRAS**
 Harvey rat sarcoma viral oncogene homolog

dbSNP ID : [104894230](#)
 Cytoband : p15.5
 COSMIC ID : [484](#) [Validate]
 99915
 Position : chr11:534288 [IGV] [UCS]
 HGMD : [CM081305](#)

Classification : [Uncertain Significance](#)
 Gene Region : Exonic
 Protein Variant : p.G12D
 Transcript Variant : c.35G>A
 Translation : missense
 Impact

SIFT Function : Damaging
 Prediction

PolyPhen-2
 Function : Benign

Filter Cascade

Variants	Genes
46076	9648

↓

× Predicted Deleterious

532	505
-----	-----

↓

× Genetic Analysis

16	11
----	----

↓

× Biological Context

5	2
---	---

Recalculate when filters change

Add Filter

Edit Columns Export Create List Search gene, chr, or dbSNP 5 variants

Chr...	Position	Gene Region	Gene Symbol	Protein Variant	Case Samples	Control Sample
11	534288	Exonic	HRAS	p.G12D	---	---

Name	p-value	#Genes	#Variants	#Cases	%Cases	#Controls	%Controls
Mdm2-Tp53-ubiquitin	1.680E-4	1	4	4	80	0	0
Mdm2-Tp53-Mdm4	2.520E-4	1	4	4	80	0	0
IkB-Tp53	4.200E-4	1	4	4	80	0	0
Ras	5.039E-4	1	1	2	40	0	0
Hd-neuronal intranuclear inclusions	1.260E-3	1	4	4	80	0	0

Variant: chr11 | 534288 | SNV

View: [More Details](#)
[Path to Phenotype](#)
[Variant Findings \(60\)](#)
[Pharmacogenetics \(375\)](#)

Gene Symbol: **HRAS**
 Harvey rat sarcoma viral oncogene homolog

dbSNP ID: [104894230](#)
 Cytoband: p15.5
 COSMIC ID: [484](#) [Validate]
 99915
 Position: [chr11:534288](#) [IGV] [UCS]
 HGMD: [CM081305](#)

Classification: [Uncertain Significance](#)
 Gene Region: Exonic
 Protein Variant: p.G12D
 transcript Variant: c.35G>A
 Translation Impact: missense
 SIFT Function Prediction: Damaging
 PolyPhen-2 Function: Benign

Filter Cascade

Variants	Genes
46076	9648

↓

Confidence

44561	9564
-------	------

↓

Common Variants

8020

↓

Predicted Deletions

532

↓

Genetic Analysis

16

↓

Biological Context

5

Recalculate when []

Add Filter []

Legend [show]

Chr...	Position	Gene Region	Gene Symbol	Protein Variant	Case Samples	Control Sample
11	534288	Exonic	HRAS	p.G12D	-- -- --	-- -- --
17	7577099	Exonic	TP53	p.R121K, p.R148G	-- -- --	-- -- --

Variant: chr11 | 534288 | SNV

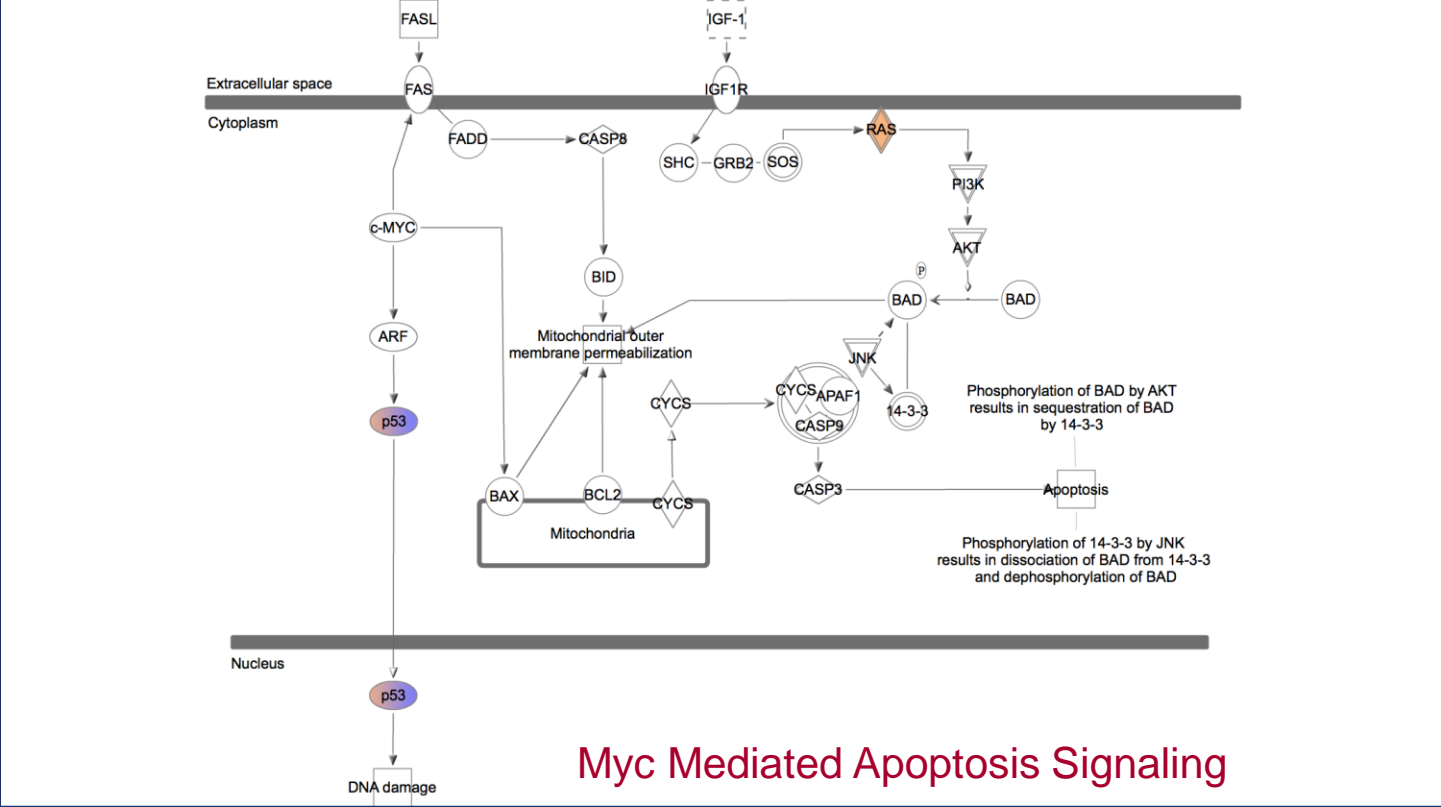
View: [More Details](#) [Path to Phenotype](#) [Variant Findings \(60\)](#) [Pharmacogenetics \(375\)](#)

HRAS
Survey rat sarcoma viral oncogene homolog

4894230
5.5
4 [Validate]
915
chr11:534288 [IGV] [UCS]
1081305

Uncertain Significance

Exonic
p.G12D
c.35G>A
missense
Damaging
Benign



Myc Mediated Apoptosis Signaling

Filter Cascade

Variants	Genes
46076	9648
↓	
Confidence	
44561	9564
↓	
Common Variants	
8020	3152
↓	
Predicted Deleterious	
532	505
↓	
Genetic Analysis	
16	11
↓	
Biological Context	
5	2

Recalculate when filters change

Add Filter

Legend [show]

Edit Columns Export Create List Search gene, chr, or dbSNP 5 variants

Chr...	Position	Gene Region	Gene Symbol	Protein Variant	Case Samples	Control Sample
11	534288	Exonic	HRAS	p.G12D	-- -- --	-----
17	7577099	Exonic	TP53	p.R121K, p.R148	-----	-----
17	7577534	Exonic	TP53	p.R117S, p.R210	-----	-----

Variant: chr11 | 534288 | SNV

View : [More Details](#)
[Path to Phenotype](#)
[Variant Findings \(60\)](#)
[Pharmacogenetics \(375\)](#)

Name	p-value	#Genes	#Variants	#Cases	%Cases	#Controls	%Controls
arrest in G1/S phase transition of embryonic cell lin	1.058E-8	2	5	5	100	0	0
arrest in developmental process of kidney cell lines	3.528E-8	2	5	5	100	0	0
arrest in growth of kidney cell lines	3.528E-8	2	5	5	100	0	0
arrest in G1/S phase transition of fibroblasts	5.292E-8	2	5	5	100	0	0
arrest in G1/S phase transition of connective tissue	7.409E-8	2	5	5	100	0	0
G1/S phase transition of embryonic cell lines	7.409E-8	2	5	5	100	0	0
arrest in cell cycle progression of breast cell lines	9.878E-8	2	5	5	100	0	0
senescence of epidermal cells	9.878E-8	2	5	5	100	0	0
senescence of dermal cells	1.588E-7	2	5	5	100	0	0
cytostasis of epidermal cells	1.588E-7	2	5	5	100	0	0
cytostasis of dermal cells	1.588E-7	2	5	5	100	0	0

SIFT Function : Damaging
 Prediction

PolyPhen-2
 Function : Benign

Ingenuity Variant Analysis

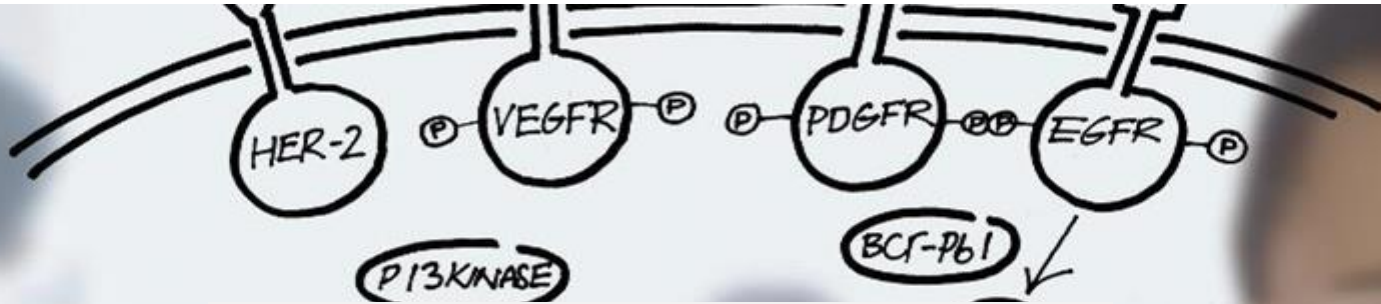
- Easy to use, allows scientific iteration
 - Filter variants, real-time, based on both standard and proprietary criteria

- Provides variant filtering using public and proprietary databases
 - Allele Frequency Community

- Powerful tools for genetic and statistical tests

- Relate variants to literature and biology
 - Narrow-down, discover, and prioritize variants based on literature findings, pathway and biological associations

- Integrate variants with GWAS, gene expression, or other gene or positional data using BED files



Questions?



Thank You!!

Dev Mistry, Ph.D.
Field Applications Scientist
Devendra.Mistry@qiagen.com

OTHER SLIDES

Increase application performance by pre-filtering large samples sets

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

Considerations for whole-genome analysis
Please review these pre-filtering options.

To make the size of the analysis you are creating manageable, Ingenuity recommends selecting at least one of these pre-filtering options. Pre-filtering will remove the least scientifically useful data so you can focus your analysis on regions of greater interest.

- Keep only variants in Exonic regions [i](#)
- Exclude common variants that are observed with high allele frequency in public databases [show details](#)
- Keep only variants above minimum confidence standards [show details](#)

[Back](#) [Next](#)

Remove unnecessary low quality or low value variants upfront to improve analysis speed

Reduce false positives with inclusion of the ExAC database for filtering out common variants

Filter ×

Common Variants

variants that are observed in any of these populations with an allele frequency of

% in the 1000 Genomes Project

% in the ExAC

% of [NHLBI ESP exomes](#)

% in the [Allele Frequency Community](#) (includes ExAC and CGI)

OR

are present in dbSNP

* The public Complete Genomics genomes are included in the AFC

- The Exome Aggregation Consortium (ExAC)
- Exome sequencing data from a wide variety of large-scale sequencing projects
- Spans 60,706 unrelated individuals sequenced as part of various disease-specific and population genetic studies.

Faster Variant Analysis setup by grouping control samples into defined libraries

Welcome Rupert Yip | Logout | What's new? [Help](#)

[Manage](#) | [My Samples](#) | [My Analyses](#) | [Publications](#) | [My Control Libraries](#)

[Upload](#) [Refresh](#) [Share](#) [Analyze](#) [Annotate](#) [New Library](#)

Showing 82 samples

ID	Barcode	Display Name	Description
267681	PGP161	PGP161	myopia dataset
408034	LP6005636-DNA_F02	LP6005636-DNA_F02	LP6005636-DNA_F02
358322	Reportability_	Reportability_tests	
267685	PGP84	PGP84	myopia dataset
339373	somatic dem	somatic demo	
407935	LP6005815-DNA_C10	LP6005815-DNA_C10	LP6005815-DNA_C10
267687	PGP171	PGP171	myopia dataset
267692	PGP81	PGP81	myopia dataset
366070	treatment_view	treatment_view1	
267674	PGP100	PGP100	myopia dataset

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

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

Use samples from reference genome GRCh37/HG19 for this analysis. Load from prior analysis

Search samples by keyword

Name	Subject	Created
187522886	221	12/16/15 04:49 PM
187522887	222	12/16/15 04:49 PM
187522884	219	12/16/15 04:49 PM
187522885	220	12/16/15 04:49 PM
187523127	125	12/16/15 04:49 PM
187523270	126	12/16/15 04:49 PM
187523256	123	12/16/15 04:49 PM
187523078	155	12/16/15 04:49 PM
187523081	156	12/16/15 04:49 PM
187523119	169	12/16/15 04:49 PM
187523121	170	12/16/15 04:49 PM
187523057	168	12/16/15 04:49 PM
187523053	167	12/16/15 04:49 PM
187523047	165	12/16/15 04:49 PM
187523048	166	12/16/15 04:49 PM

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

187523238

187523102

187523100

187523092

187523125

187523091

187523089

PGP-60 Library

Use individual samples

Back
Next

Export directly to IPA with inferred gain/loss-of-function values

Project Manager

A-Z SORT SEARCH REFRESH

- My Projects
 - IVA Datasets**
 - EEC From JN
 - RNAseq Cluadin Low
 - Rupert_IPA_Test
 - Variant Analysis
 - Training Project
 - Ingenuity KEGG gene lists
 - Human Genes Chromosomal Location
 - Example Analyses
 - Tissue Expression
- Shared Projects
 - Projects Shared with Others
 - Projects Shared with Me
- Libraries

Annotated Dataset: 2016-Feb-02:11:39:27-CGI Breast Cancer Tumor Cell Line-Genetic Analysis

Preview Dataset 2016-Feb-02:11:39:27-CGI Breast Cancer Tumor Cell Line-Genetic Analysis Observation: cases (66)

Mapped IDs (66) Unmapped IDs (0) All IDs (66)

ADD TO MY PATHWAY ADD TO MY LIST CREATE DATASET CUSTOMIZE TABLE

Variant AC...	Variant Gai...	ID	Notes	Sy...	Entrez Gene...	Location	Type(s)	Drug(s)
0.000	0.000	ABHD16A		ABHD16A	abhydrolase dom...	Other	other	
0.000	0.000	ADAT3		ADAT3	adenosine deami...	Nucleus	enzyme	
0.000	0.000	ANKRD29		ANKRD29	ankyrin repeat do...	Other	other	
0.000	↓ -1.000	ANKRD36C		ANKRD36C	ankyrin repeat do...	Other	other	
0.000	↓ -1.000	APC2		APC2	adenomatosis po...	Cytoplasm	enzyme	
0.000	↓ -1.000	BCORL1		BCORL1	BCL6 corepressor...	Nucleus	transcription reg...	
0.000	0.000	C12orf40		C12orf40	chromosome 12 ...	Other	other	
0.000	0.000	CIC		CIC	capicua transcrip...	Other	other	
0.000	0.000	CLCN3		CLCN3	chloride channel...	Plasma Membrane	ion channel	
0.000	0.000	CLDN25		CLDN25	claudin 25	Plasma Membrane	other	
0.000	↓ -1.000	CLTC		CLTC	clathrin, heavy c...	Plasma Membrane	other	
0.000	↓ -1.000	COG3		COG3	component of oli...	Cytoplasm	transporter	
0.000	0.000	COL14A1		COL14A1	collagen, type XI...	Extracellular Space	other	collagenase clost...
0.000	↓ -1.000	CPSF3		CPSF3	cleavage and pol...	Nucleus	enzyme	
0.000	↓ -1.000	DHX40		DHX40	DEAH (Asp-Glu-...	Other	enzyme	
0.000	↓ -1.000	EHMT1		EHMT1	euchromatic hist...	Nucleus	transcription reg...	
0.000	0.000	FAM134B		FAM134B	family with sequ...	Cytoplasm	other	
0.000	0.000	HELQ		HELQ	helicase, POLQ-li...	Nucleus	enzyme	

0 / 66

Notes:
 "D" - Duplicates. Gene/Protein/Chemical identifiers marked with an asterisk indicate that multiple identifiers in the dataset file map to a single gene/chemical in the Global Molecular Network.
 "O" - Override molecules. Gene/Protein/Chemical identifiers marked as "Override" are displayed with italic text.
 "A" - Gene/Protein/Chemical ID marked as Absent. The gene/protein/chemical will not be used as a focus molecule or appear in networks unless you also explicitly override this flag with the Override column.

EDIT DATASET SETTINGS ANALYZE/FILTER DATASET CLOSE

www.allelefrequencycommunity.org

- Leverage the world's largest pool of anonymized allele frequency data
- **Reduces false positives** in analyses by removing variants that are commonly seen in the general population
- Contains Whole Exome AND Whole Genome data
- Better representation of Insertions and Deletions
- **Larger than ANY other public resource**
 - AFC launched with 70,000 samples with >8,000 as whole genomes
 - 12x larger than Exome Variant Server data
- The initial launch version of the database already provides a 43% average false positive rate reduction in a benchmarking set of whole-genome Diagnostic Odyssey cases
- AFC will grow as more people opt-in
 - Launched on 25th February 2015 with 70,000 Samples including 8,000 Whole Genomes
 - Currently at over 100,000 samples, including over 14,000 Whole Genome samples

Ingenuity Variant Analysis

- Easy to use, allows scientific iteration
 - Filter variants, real-time, based on both standard and proprietary criteria

- Provides variant filtering using public and proprietary databases
 - Allele Frequency Community

- Powerful tools for genetic and statistical tests

- Relate variants to literature and biology
 - Narrow-down, discover, and prioritize variants based on literature findings, pathway and biological associations

- Integrate variants with GWAS, gene expression, or other gene or positional data using BED files