Precision medicine: NGS variant analysis and interpretation for translational research

Selecting the most relevant variants: How to filter

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INSTITUTO GULBENKIAN DE CIÊNCIA

Additional annotations

chromosome	PolyPhen score	VEP's COSMIC ID
location	Condel effect	other IDs
mutation	Condel score	variation type
gene	SIFT effect	HGVS cDNA
feature	SIFT score	HGVS protein
feature type	gene HGNC	GMAF 1000 genomes
consequence	protein position	GMAF 1000 genomes percentage
	amino acids	ExAC percentage
PolyPhen effect	dbSNP ID	ExAC NFE percentage

Annotations from VEP

Additional annotations

chromosome	PolyPhen score	VEP's COSMIC ID	COSMIC original ID
location	Condel effect	other IDs	Pfam
mutation	Condel score	variation type	Uniprot
gene	SIFT effect	HGVS cDNA	Interpro
feature	SIFT score	HGVS protein	
feature type	gene HGNC	GMAF 1000 genomes	
consequence	protein position	GMAF 1000 genomes percentage	
APPRIS category	amino acids	ExAC percentage	
PolyPhen effect	dbSNP ID	ExAC NFE percentage	

Annotations from VEP

Enrichment of VEP annotations

Additional annotations

chromosome	PolyPhen score	VEP's COSMIC ID	COSMIC original ID	ClinVar disease
location	Condel effect	other IDs	Pfam	ClinVar clinical significance
mutation	Condel score	variation type	Uniprot	Homopolymer
gene	SIFT effect	HGVS cDNA	Interpro	Repeats
feature	SIFT score	HGVS protein	TumorPortal	CCLE gene
feature type	gene HGNC	GMAF 1000 genomes	Role of the gene in tumorgenesis	Frequency of gene in COSMIC
consequence	protein position	GMAF 1000 genomes percentage	KEGG data	Frequency of mutation in COSMIC
APPRIS category	amino acids	ExAC percentage	KEGG path ID	Consensual role
PolyPhen effect	dbSNP ID	ExAC NFE percentage	ClinVar ID	VSCORE

Annotations from VEP

Enrichment of VEP annotations

Annotations from other sources

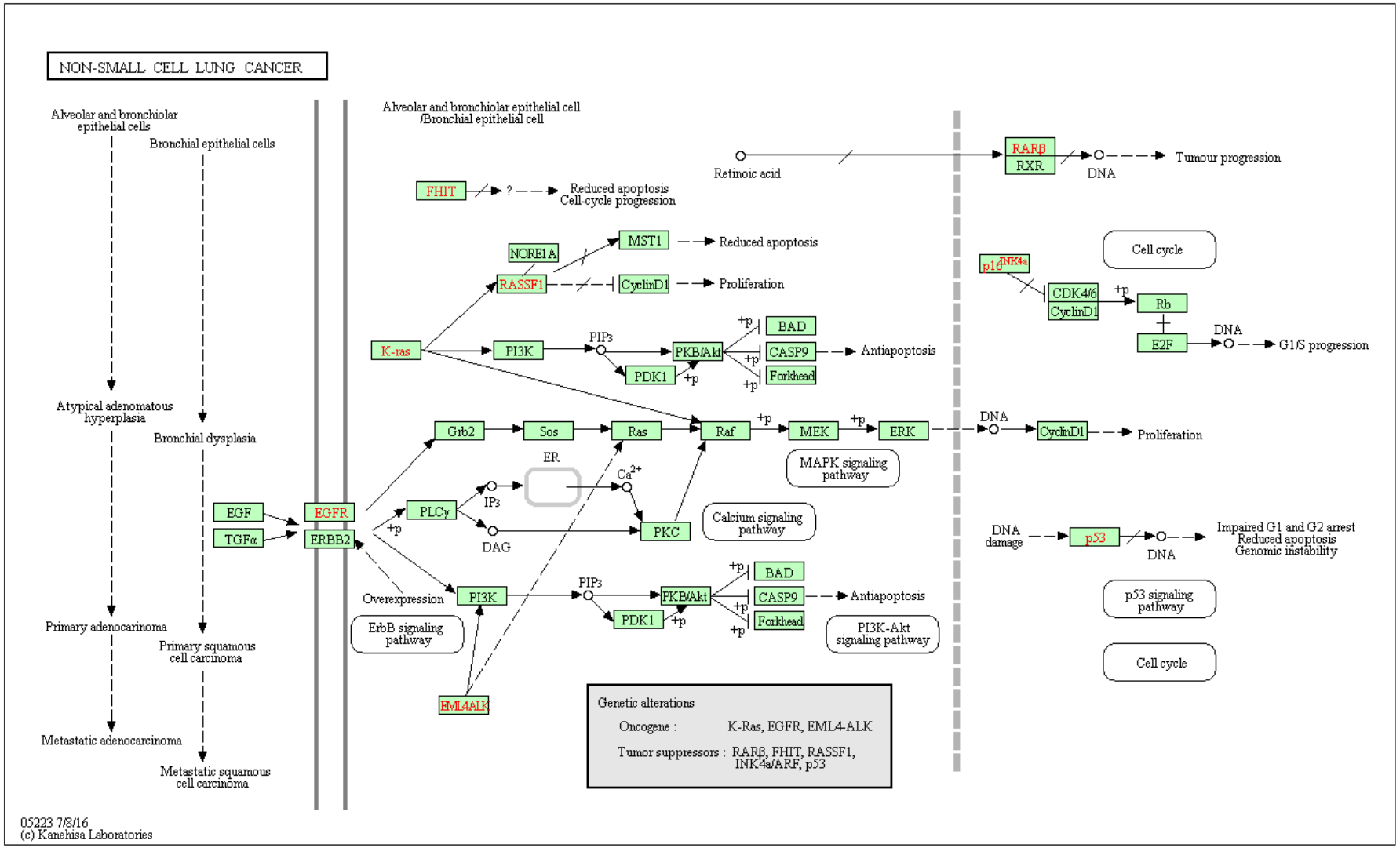
KEGG pathways

http://www.genome.jp/kegg/pathway.html

KEGG PATHWAY Database

Wiring diagrams of molecular interactions, reactions, and relations

1. Metabolism
2. Genetic Information Processing
3. Environmental Information Processing
4. Cellular Processes
5. Organismal Systems
6. Human diseases
7. Drug development (structural relations between compounds)



ClinVar

http://www.ncbi.nlm.nih.gov/clinvar/

	Variation *Location*	Gene(s)	Condition(s)	Frequency	Clinical significance (Last reviewed)	Review status
19.	NM_001005862.2(**ERBB2**):c.1376C>T (p.Pro459Leu) *GRCh37:* Chr17:37872145 *GRCh38:* Chr17:39715892	**ERBB2**	not specified	GMAF:0.00040(T)	not provided (Sep 19, 2013)	no assertion provided
20.	NM_001005862.2(**ERBB2**):c.1703C>A (p.Ala568Asp) *GRCh37:* Chr17:37873628 *GRCh38:* Chr17:39717375	**ERBB2**	not specified		not provided (Sep 19, 2013)	no assertion provided
21.	NM_001005862.2(**ERBB2**):c.1870A>G (p.Ile624Val) *GRCh37:* Chr17:37879585 *GRCh38:* Chr17:39723332	**ERBB2**	**ERBB2** POLYMORPHISM, not specified	GO-ESP:0.00707(G) GMAF:0.00260(G)	Benign (Feb 1, 1993)	no assertion criteria provided
22.	NM_001005862.2(**ERBB2**):c.1873A>G (p.Ile625Val) *GRCh37:* Chr17:37879588 *GRCh38:* Chr17:39723335	**ERBB2**	**ERBB2** POLYMORPHISM, not specified	GO-ESP:0.16854(G) GMAF:0.12140(G)	Benign (Feb 1, 1993)	no assertion criteria provided
23.	NM_001005862.2(**ERBB2**):c.2173_2174delTTinsCC (p.Leu725Pro) *GRCh37:* Chr17:37880219-37880220 *GRCh38:* Chr17:39723966-39723967	**ERBB2**	Adenocarcinoma of lung		Pathogenic (Sep 30, 2004)	no assertion criteria provided

UniProt additional information

UniProtKB - P00533 (EGFR_HUMAN)

Protein | **Epidermal growth factor receptor**

Gene | **EGFR**

Organism | *Homo sapiens (Human)*

Status | Reviewed - Annotation score: ●●●●● - Experimental evidence at protein level[i]

PTM / Processing[i]

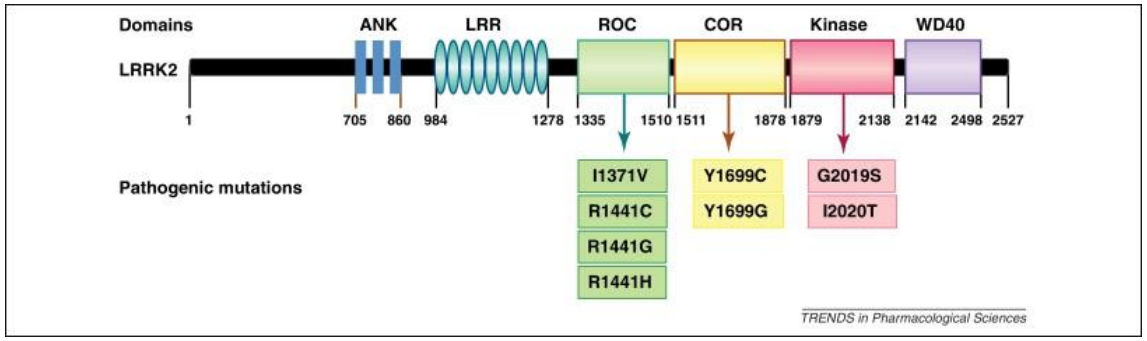
Molecule processing

Feature key	Position(s)	Length	Description	Graphical view	Feature identifier	Actions
Signal peptide[i]	1 – 24	24	2 Publications			Add BLAST
Chain[i]	25 – 1210	1186	Epidermal growth factor receptor		PRO_0000016665	Add BLAST

Amino acid modifications

Feature key	Position(s)	Length	Description	Graphical view	Feature identifier	Actions
Disulfide bond[i]	31 ↔ 58					
Glycosylation[i]	56 – 56	1	N-linked (GlcNAc...) (complex); atypical; partia 5 Publications		CAR_000227	
Glycosylation[i]	73 – 73	1	N-linked (GlcNAc...); atypica 1 Publication			
Glycosylation[i]	128 – 128	1	N-linked (GlcNAc...) 3 Publications			
Disulfide bond[i]	157 ↔ 187					
Glycosylation[i]	175 – 175	1	N-linked (GlcNAc...) 5 Publications			
Disulfide bond[i]	190 ↔ 199					
Disulfide bond[i]	194 ↔ 207					
Glycosylation[i]	196 – 196	1	N-linked (GlcNAc...) 4 Publications			

Additional domains information: pfam and Interpro



EMBL-EBI InterPro

EMBL-EBI Pfam

Protein

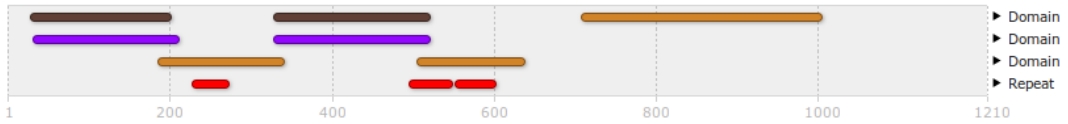
Epidermal growth factor receptor (P00533)

Accession	P00533 (EGFR_HUMAN)
Species	Homo sapiens (Human)
Length	1,210 amino acids (complete)

Protein family membership

Tyrosine protein kinase, EGF/ERB/XmrK receptor (IPR016245)

Domains and repeats



Source	Domain	Start	End
sig_p	n/a	1	24
low_complexity	n/a	6	24
Pfam	Recep_L_domain	57	168
Pfam	Furin-like	177	338
Pfam	Recep_L_domain	361	481
Pfam	GF_recep_IV	505	637
transmembrane	n/a	646	667
low_complexity	n/a	650	665
low_complexity	n/a	674	691
Pfam	Pkinase_Tyr	712	968

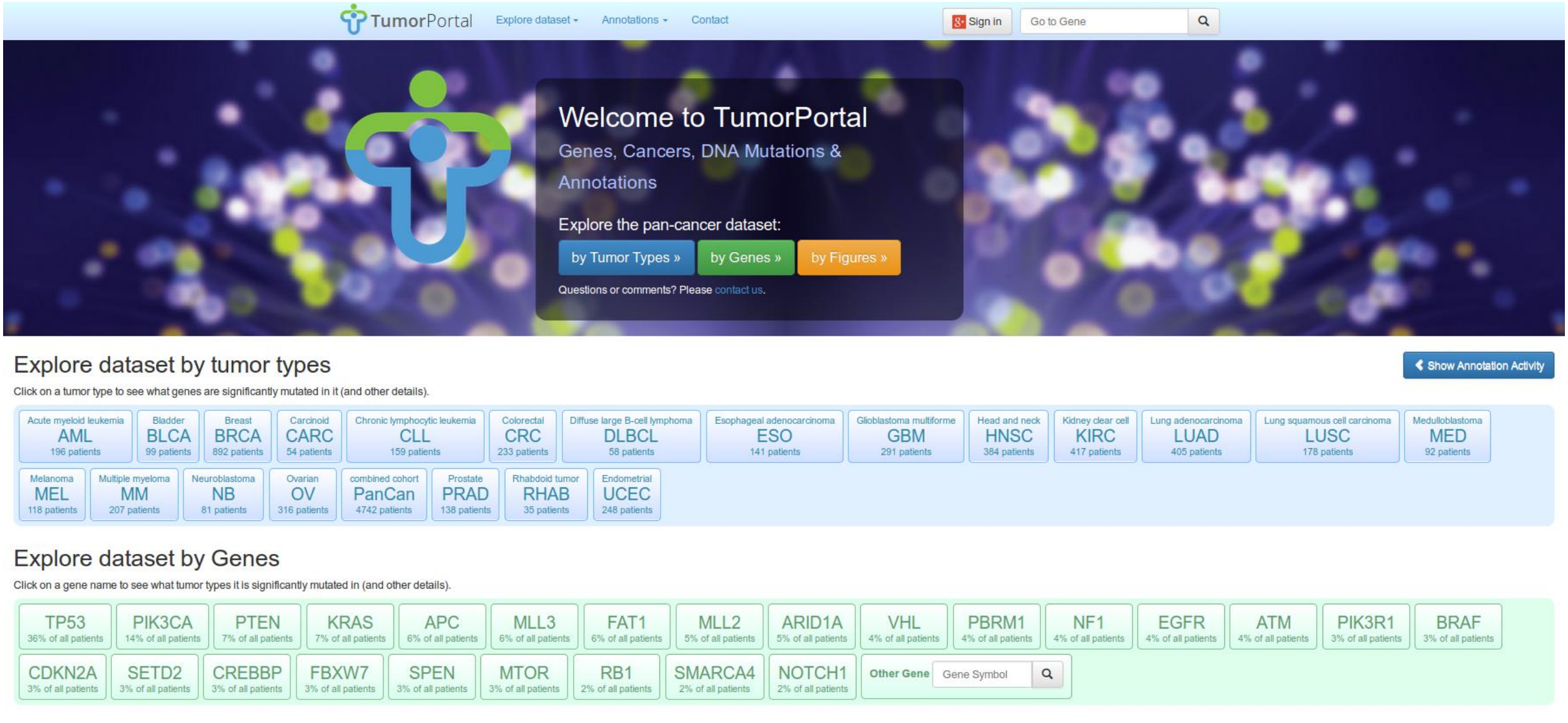
Description:	Epidermal growth factor receptor EC=2.7.10.1
Source organism:	Homo sapiens (Human) (NCBI taxonomy ID 9606) View Pfam proteome data.
Length:	1210 amino acids
Reference Proteome:	✓

Specific cancer information

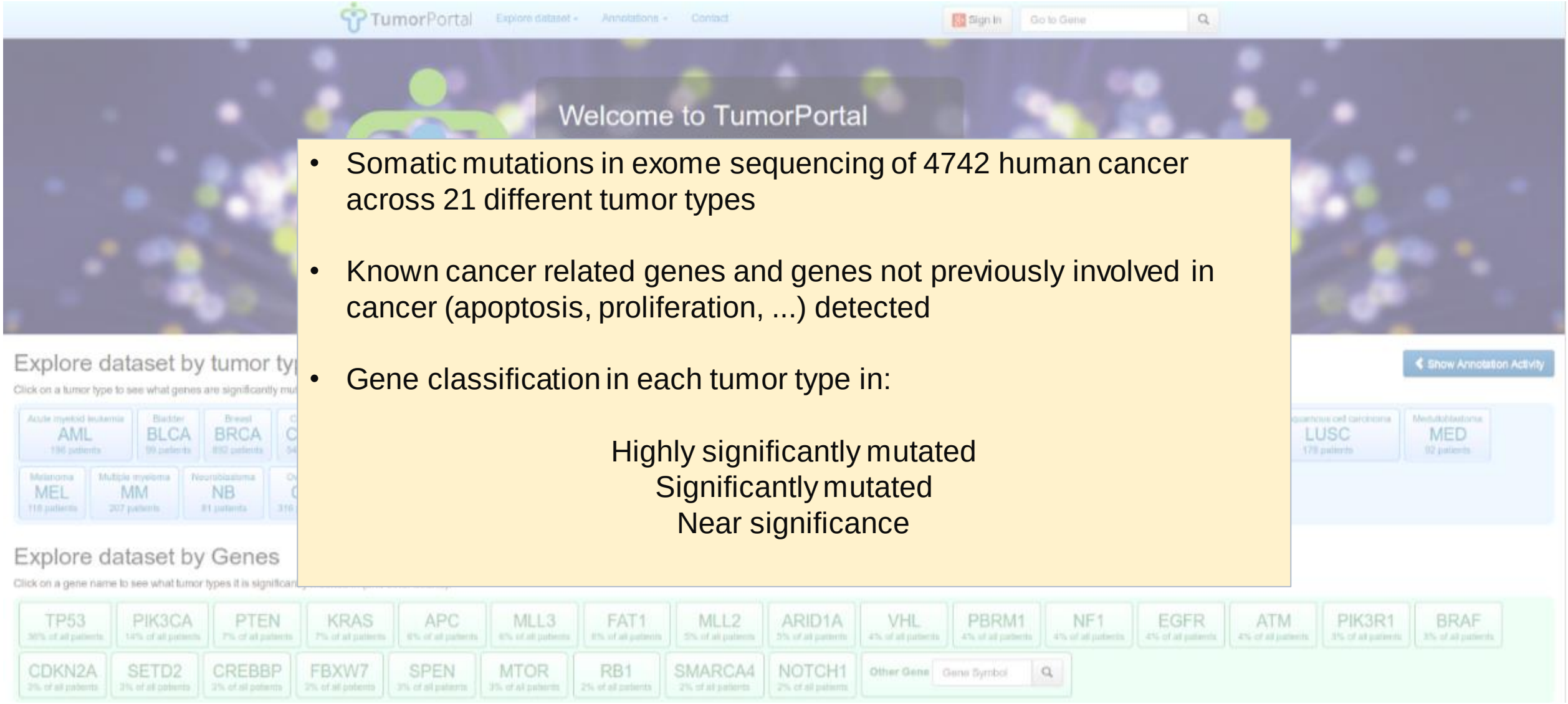
- Relevance of the gene in carcinogenesis
  - TumorPortal
  - CCLE
  - COSMIC
- Frequency of the variant/gene in cancer
  - COSMIC
- Role of the gene in carcinogenesis (Oncogene or Tumor Suppressor)
  - COSMIC
  - oncodriveROLE

TumorPortal

http://www.tumorportal.org/



TumorPortal



- Somatic mutations in exome sequencing of 4742 human cancer across 21 different tumor types
- Known cancer related genes and genes not previously involved in cancer (apoptosis, proliferation, ...) detected
- Gene classification in each tumor type in:

Highly significantly mutated
Significantly mutated
Near significance

CCLE

Cancer Cell Line Encyclopedia (CCLE)

The Cancer Cell Line Encyclopedia (CCLE) project is an effort to conduct a detailed genetic characterization of a large panel of human cancer cell lines. The CCLE provides public access analysis and visualization of DNA copy number, mRNA expression, mutation data and more, for 1000 cancer cell lines.

Contact: ccle-help@broadinstitute.org

Data Info:

URL: http://www.broadinstitute.org/ccle
Description:
A link to the CCLE portal

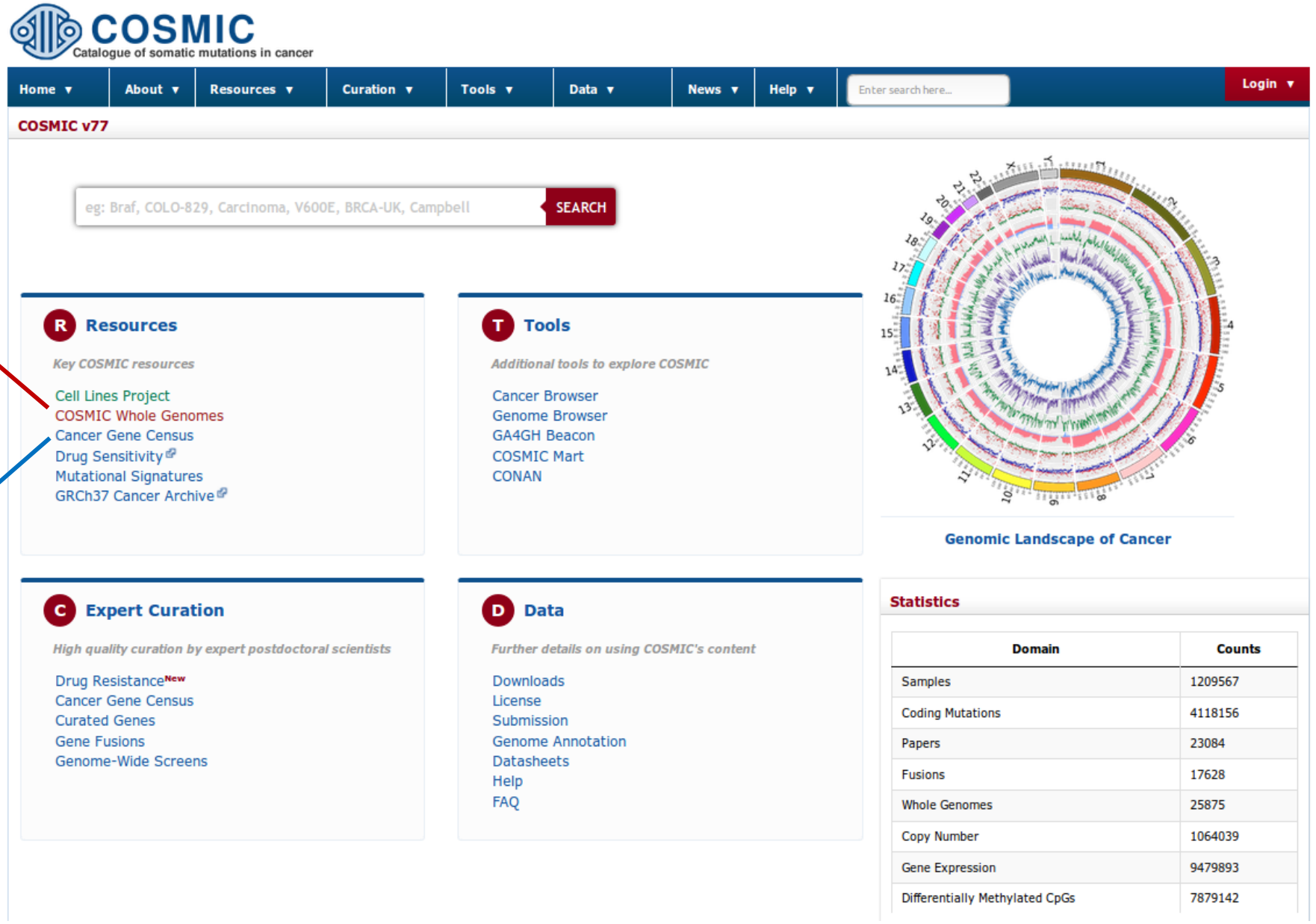
Publication Info:

URL: http://www.nature.com/nature/journal/v483/n7391/full/nature11003.html
Date: 3/29/2012
Notes:
Barretina, Caponigro, Stransky et al. The Cancer Cell Line Encyclopedia enables predictive modelling of anticancer drug sensitivity. Nature. 2012 Mar 28;483(7391):603-7. doi: 10.1038/nature11003.

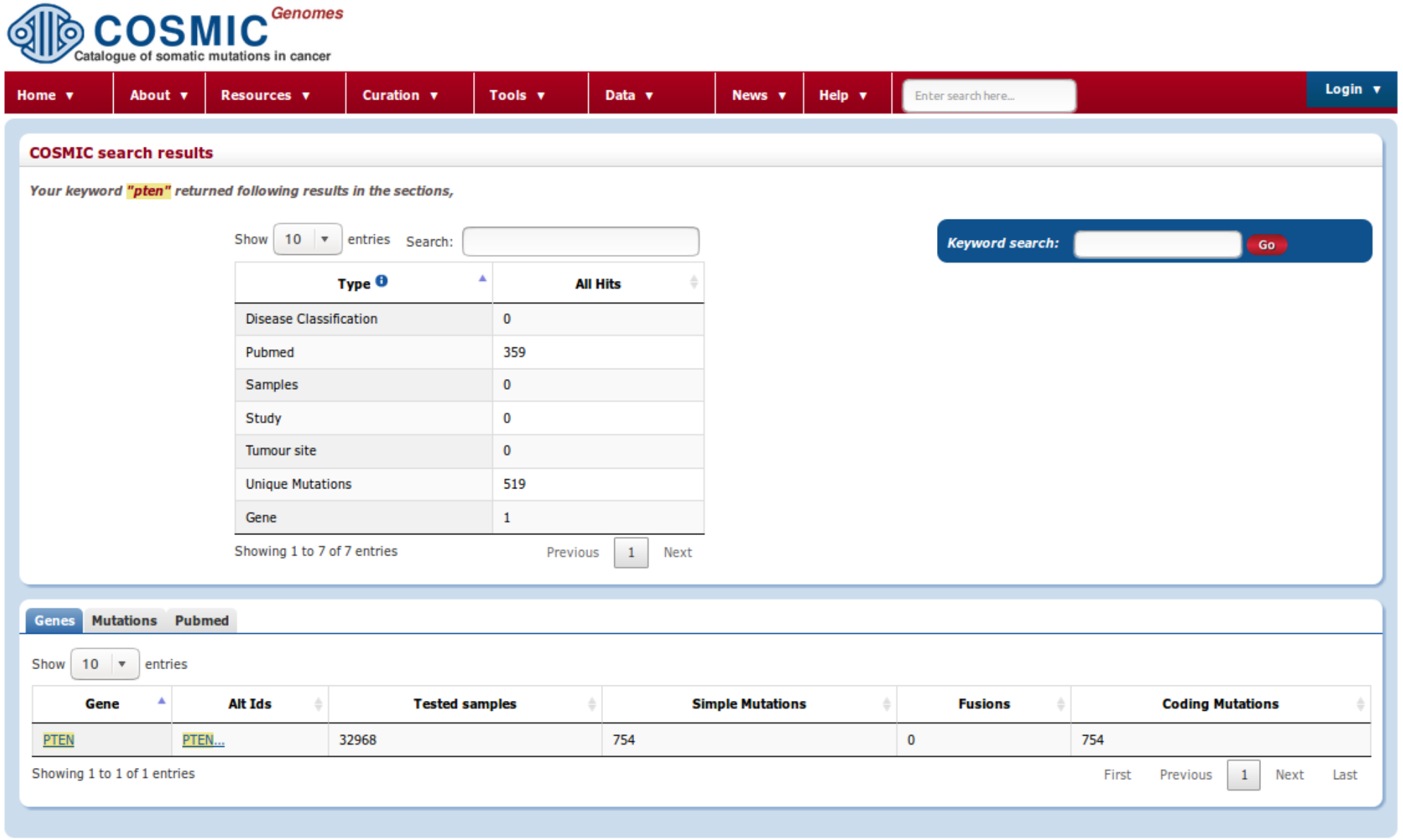
COSMIC additional information

variant/gene frequency in database

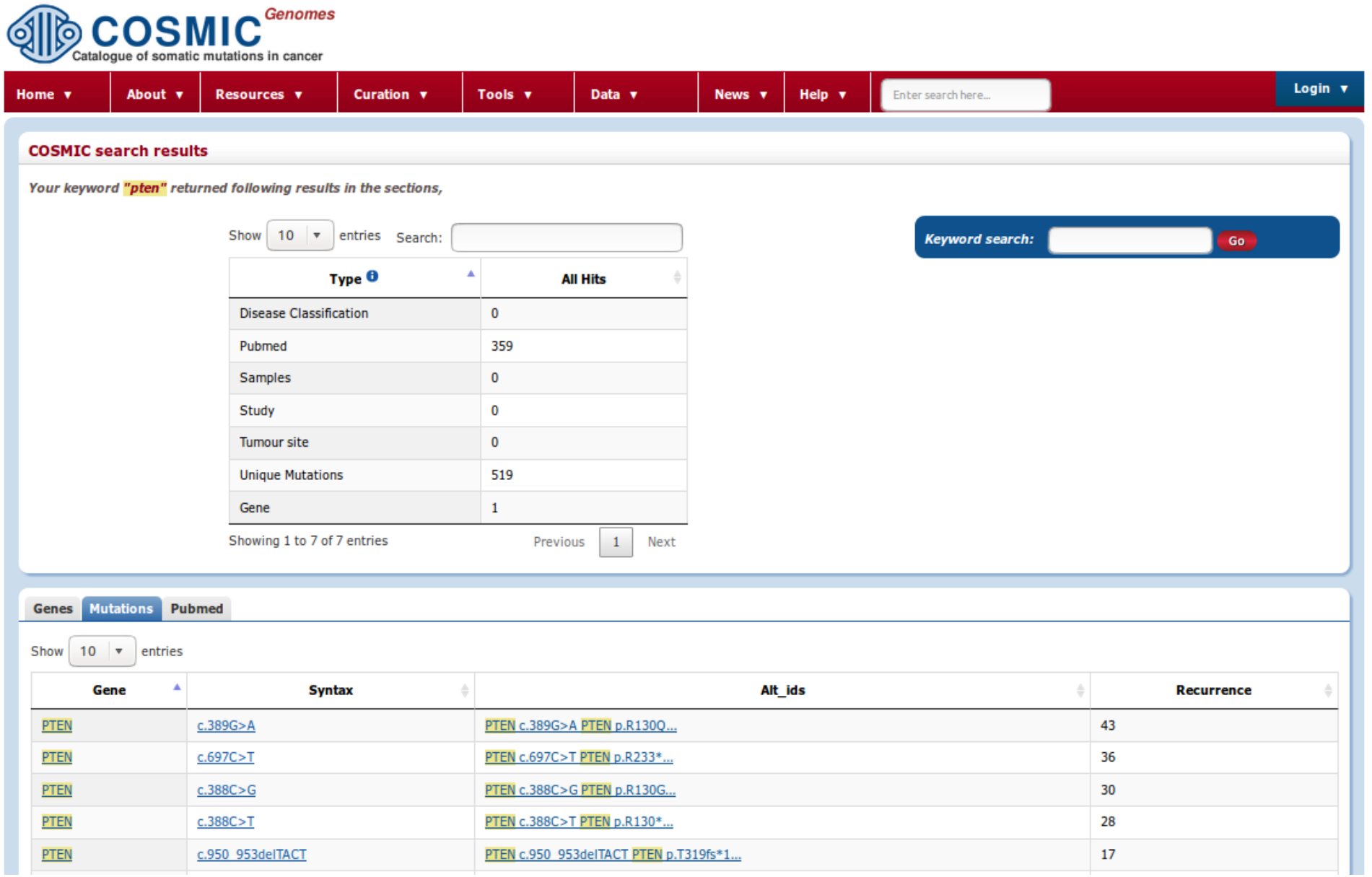
cancer genes information (e.g. role of the gene)



Variant / gene frequency in COSMIC



Variant / gene frequency in COSMIC



COSMIC Genomes
Catalogue of somatic mutations in cancer

Home ▾ | About ▾ | Resources ▾ | Curation ▾ | Tools ▾ | Data ▾ | News ▾ | Help ▾ | Enter search here... | Login ▾

COSMIC search results

Your keyword "pten" returned following results in the sections,

Show 10 entries Search:

Keyword search: Go

Type	All Hits
Disease Classification	0
Pubmed	359
Samples	0
Study	0
Tumour site	0
Unique Mutations	519
Gene	1

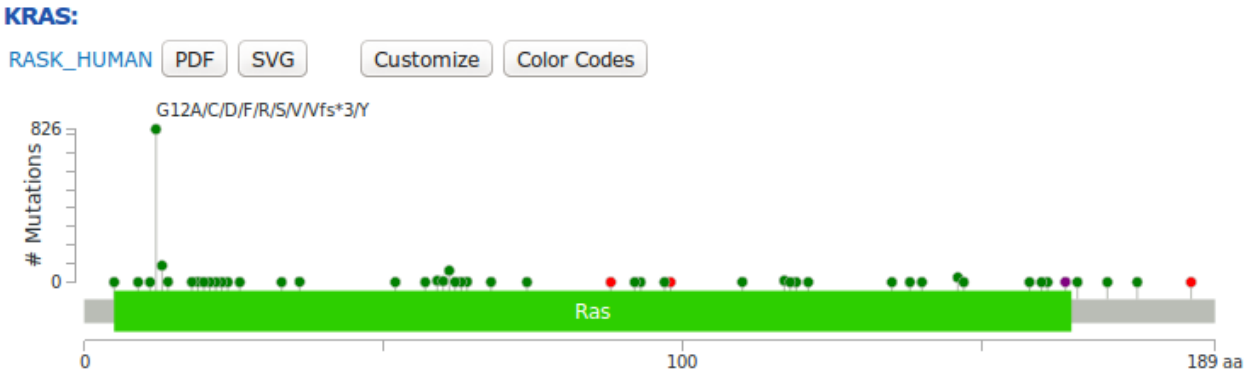
Showing 1 to 7 of 7 entries Previous 1 Next

Genes | Mutations | Pubmed

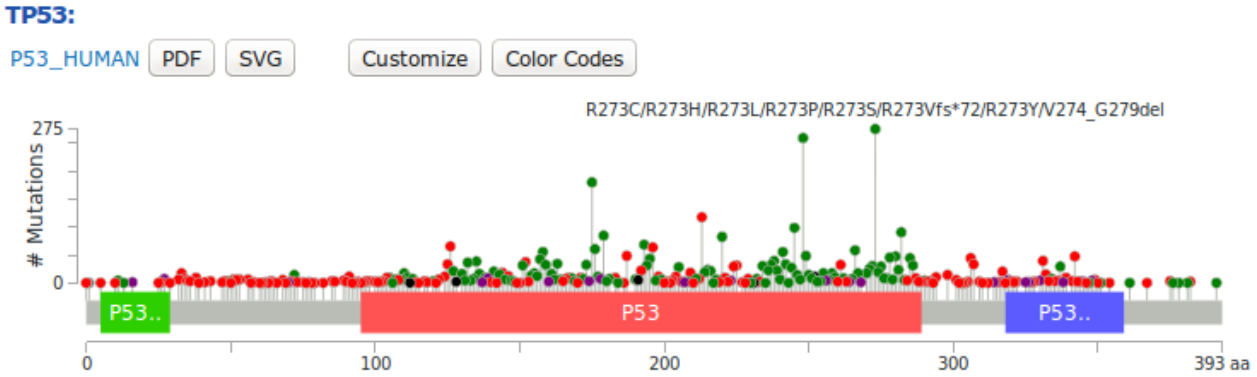
Show 10 entries

Gene	Syntax	Alt_ids	Recurrence
PTEN	c.389G>A	PTEN c.389G>A PTEN p.R130Q...	43
PTEN	c.697C>T	PTEN c.697C>T PTEN p.R233*...	36
PTEN	c.388C>G	PTEN c.388C>G PTEN p.R130G...	30
PTEN	c.388C>T	PTEN c.388C>T PTEN p.R130*...	28
PTEN	c.950_953delTACT	PTEN c.950_953delTACT PTEN p.T319fs*1...	17

Role of the gene as ONC or TSG



ONCOGENE



TUMOR SUPPRESSOR GENE

How frequencies can be interpreted?
How genes can be actioned for therapy?

Role of the gene as ONC or TSG

COSMIC
Catalogue of somatic mutations in cancer

http://cancer.sanger.ac.uk/census/

The cancer Gene Census is an ongoing effort to catalogue those genes for which mutations have been causally implicated in cancer. The original census and analysis was published in Nature Reviews Cancer and supplemental analysis information related to the paper is also available.

The census is not static but rather is updated regularly/as needed. In particular we are grateful to Felix Mitelman and his colleagues in providing information on more genes involved in uncommon translocations in leukaemias and lymphomas. Currently, more than 1% of all human genes are implicated via mutation in cancer. Of these, approximately 90% have somatic mutations in cancer, 20% bear germline mutations that predispose to cancer and 10% show both somatic and germline mutations.

Show 10 entries Export: CSV TSV Search:

Gene Symbol	Name	Entrez GeneId	Genome Location	Chr Band	Somatic	Germline	Tumour Types(Somatic)	Tumour Types(Germline)	Cancer Syndrome	Tissue Type	Molecular Genetics
BRAF	v-raf murine sarcoma viral oncogene homolog B1	673	7:140734597-140924703	7q34	yes		melanoma; colorectal; papillary thyroid; borderline ovarian; NSCLC; cholangiocarcinoma; pilocytic astrocytoma; Spitzoid tumour; pancreas acinar carcinoma; melanocytic nevus; prostate; gastric			E; O	Dom
BRCA1	familial breast/ovarian cancer gene 1	672	17:43045678-43124096	17q21	yes	yes	ovarian	breast; ovarian	hereditary breast/ovarian cancer	E	Rec
BRCA2	familial breast/ovarian cancer gene 2	675	13:32316461-32398770	13q12	yes	yes	breast; ovarian; pancreatic	breast; ovarian; pancreatic; leukaemia (FANCB; FANCD1)	hereditary breast/ovarian cancer	L; E	Rec

ONC

TSG

Role of the gene as ONC or TSG

http://bg.upf.edu/oncodrive-role/

OncodriveROLE

Classifying cancer driver genes into Loss of Function and Activating roles.

We developed the machine-learning based approach OncodriveROLE to classify cancer driver genes into to Activating or Loss of Function roles for cancer gene development. Here you can download the code of the method, and browse the results of applying OncodriveROLE to two recently published list of driver genes (HCDs and Cancer5000) in the respective tabs Plots, Gene classification and performance. You may adjust the cut-offs with the sliders to the left, download the results according to the selected cut-offs or directly download the classifier to use with your own data. For further information please refer to the manuscript.

Loss of function cutoff:

0 0.3 1

Activating cutoff:

0 0.7 1

Cancer driver list

- Cancer5000
- HCD

Download classification

Download & Usage | Performance and plots | Gene classification | Validation

25 records per page

Search:

ENSG	SYM	oncodriveROLE	Value
ENSG00000000971	CFH	Activating	0.9180
ENSG00000009307	CSDE1	Loss of function	0.2515
ENSG00000012048	BRCA1	Loss of function	0.0020
ENSG00000019991	HGF	No class	0.4235

Filtering process

Once variants have been annotated we can remove the non likely relevant using the annotation information.

We can select manually those that seem more relevant according to a **set of criteria**.

A useful tool for the selection is the prioritization based on a **score calculation** computed from selected annotations. This provides a ranked list of variants with the most relevant at the top.

Components in the selection criteria and score calculation vary with the pathology or condition under study.

Filtering criteria

Remove **artifacts**

Possible artifacts

Table 1 | **Main characteristics of current NGS technologies**

Technology	Run type			Maximum read length	Quality scores	Error rates	Refs
	Single end	Paired end	Mate pair				
Illumina	Yes	Yes	Yes	300 bp	>30	0.0034–1%	59
SOLiD	Yes	Yes	Yes	75 bp	>30	0.01–1%	60
IonTorrent	Yes	Yes	No	400 bp	~20	1.78%	22
454	Yes	Yes	No	~700 bp (up to 1 kb)	>20	1.07–1.7%	53,61
Nanopore	Yes	No	No	5.4–10 kb	NA	10–40%	62–66
PacBio	Yes	No	No	~15 kb (up to 40 kb)	<10	5–10%	22,67–69

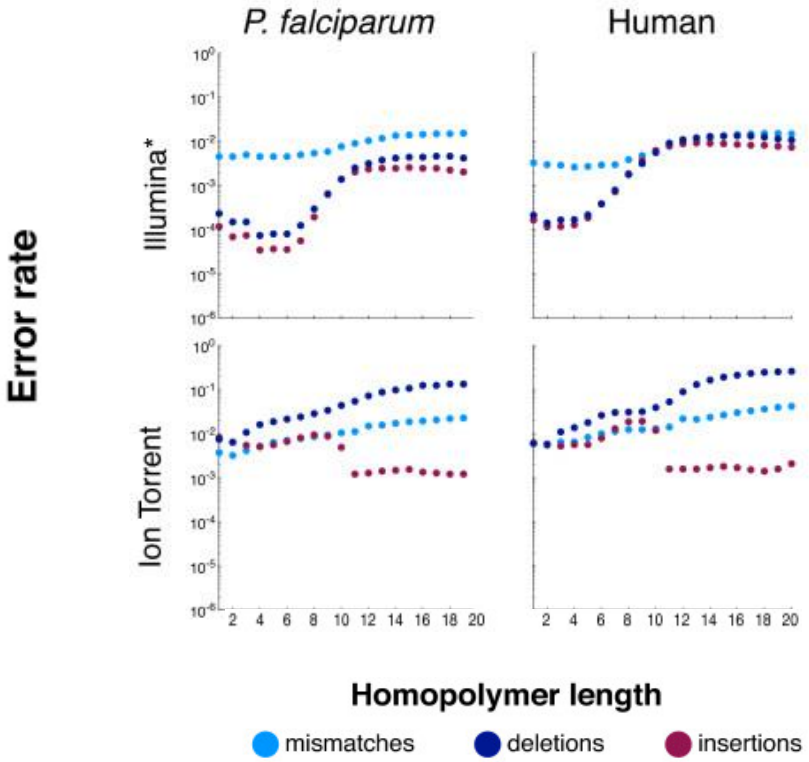
454, 454 pyrosequencing (Roche); NA, not applicable; Nanopore, Oxford Nanopore Technologies; NGS, next-generation sequencing; PacBio, Pacific Biosciences; SOLiD, sequencing by oligonucleotide ligation and detection (Thermo Fisher).

Nature Reviews Genetics 17,459–469(2016)doi:10.1038/nrg.2016.57

Sequencing strategies differ in different aspects as the error rates they produce and the kind of sequencing errors they introduce

Possible artifacts

- Base-calling errors
  - Indel errors: Rare in Illumina. Main source of errors in IonTorrent and 454.
  - Substitution errors: Dominant in Illumina and SOLiD platforms.



Adapted from: Ross *et al. Genome Biology* 2013 **14**:R51 doi:10.1186/gb-2013-14-5-r51

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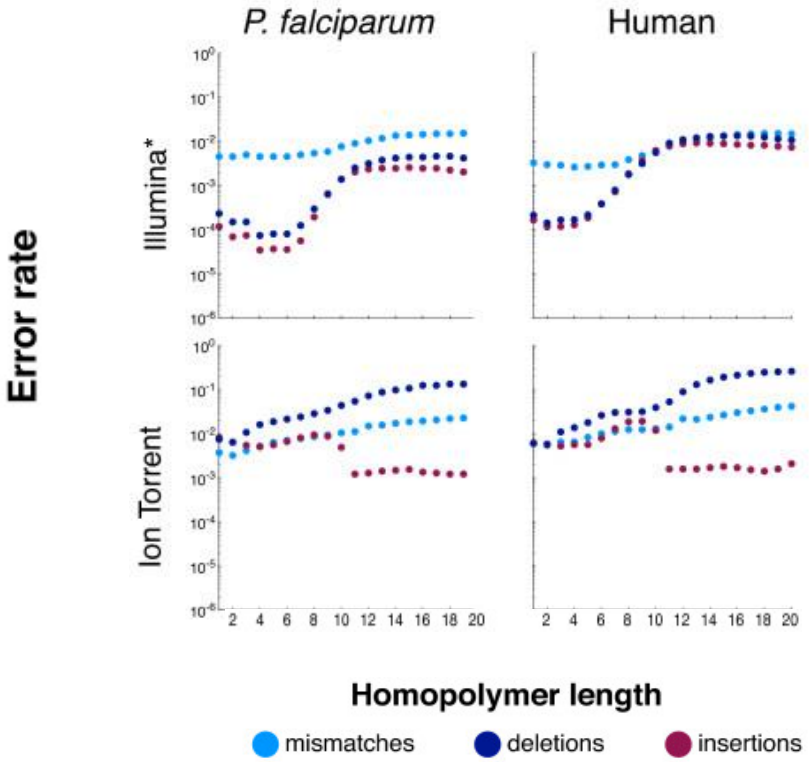


Detection

Quality filters

Homopolymeric regions

Repetition in same technology output

Check in IGV

- Repetition can indicate a polymorphism if it is present in at least a 1% of the population.
- Repetition can indicate a frequent cancer alteration if its presence is validated in multiple cancer samples.
- Otherwise, it can be an artifact (especially in genes acting as tumor suppressor)

Adapted from: Ross *et al. Genome Biology* 2013 **14**:R51 doi:10.1186/gb-2013-14-5-r51

Filtering criteria

Remove **artifacts**

- High number of repetitions without high frequency in population or in cancer samples.
- Indels located in homopolymeric regions in data from sensitive platforms to this artifact.
- Variants in positions with very low coverage.

Remove variants located in **no functional genes**: BACs, pseudogenes, …

Remove **polymorfisms**: Population frequency in 1000 Genomes project, ExAC, … >= 1% (if not interested in germline information)

Filtering criteria

Keep variants with **relevant consequences at transcriptional level**:

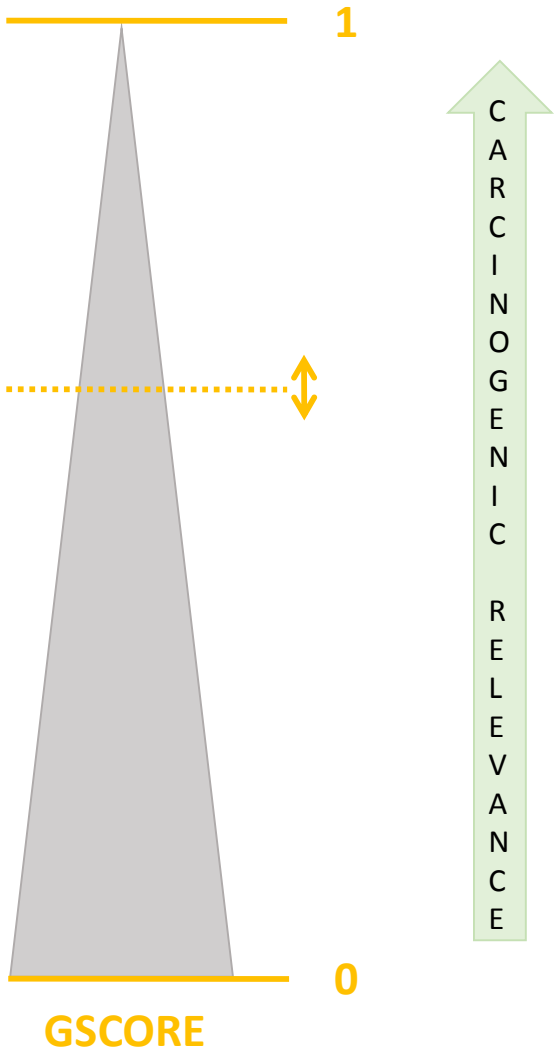
transcript_ablation | splice_donor_variant | splice_acceptor_variant | stop_gained | frameshift_variant | stop_lost | start_lost | transcript_amplification | inframe_insertion | inframe_deletion | missense_variant | protein_altering_variant | splice_region_variant | incomplete_terminal_codon_variant | stop_retained_variant

Keep variants with predicted **relevant consequence at protein level**: damaging in predictors, affecting domains

Keep variants with **clinical significance**: pathogenic ClinVar

Keep variants **relevant in the pathology**: pathogenic COSMIC, gene or variant frequently mutated in cancer, ...

Score calculation: an example



Feature	Value	Weight ONC	Weight TSG
Score prediction by PolyPhen	> 0.435	0.125/3	
Score prediction by Sift	<= 0.05	0.125/3	
Score prediction by CONDEL	> 0.468	0.125/3	
COSMIC	Pathogenic by FATHMM prediction	0.125/3	0.03125
Frequency of mutation in COSMIC	>= 100	0.125/3	
	< 100	(0.125 / 3) * (log(mutation frequency) / log(maximum mutation frequency))	
Frequency of gene in COSMIC	>= 100	0.125/3	0.03125
	< 100	(0.125 / 3) * (log(gene frequency) / log(maximum gene frequency))	0.03125 * (log(gene frequency) / log(maximum gene frequency))
VEP consequence	stop gain frameshift missense inframe insertion inframe deletion	0.125	
GMAF	< 1	0.125/2	
EXAC	< 1	0.125/2	
DOMAINS	Listed as relevant in cancer or previous last protein domain	0.125	
	Within a domain in other circumstances	0.125/2	
CLINVAR	Pathogenic	0.125	
ZYGOSITY	Homozygous	0.125	0.1875
ESSENTIALITY SCORE		0.125 * ES	

Impact

Pathogenicity

Frequencies

Impact

Frequencies

Impact

Pathogenicity

Impact

THE END

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