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	gnomAD percentage
PolyPhen effect	dbSNP ID	gnomAD 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	gnomAD percentage	
PolyPhen effect	dbSNP ID	gnomAD 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	gnomAD percentage	KEGG path ID	Consensual role
PolyPhen effect	dbSNP ID	gnomAD NFE percentage	ClinVar ID	VSCORE

Annotations from VEP

Enrichment of VEP annotations

Annotations from other sources

Pathways

- Implication of genes in biological process
- Interaction among proteins -> Therapeutic implications

- **Resources**
  - Reactome
  - KEGG (Kyoto Encyclopedia of Genes and Genomes)

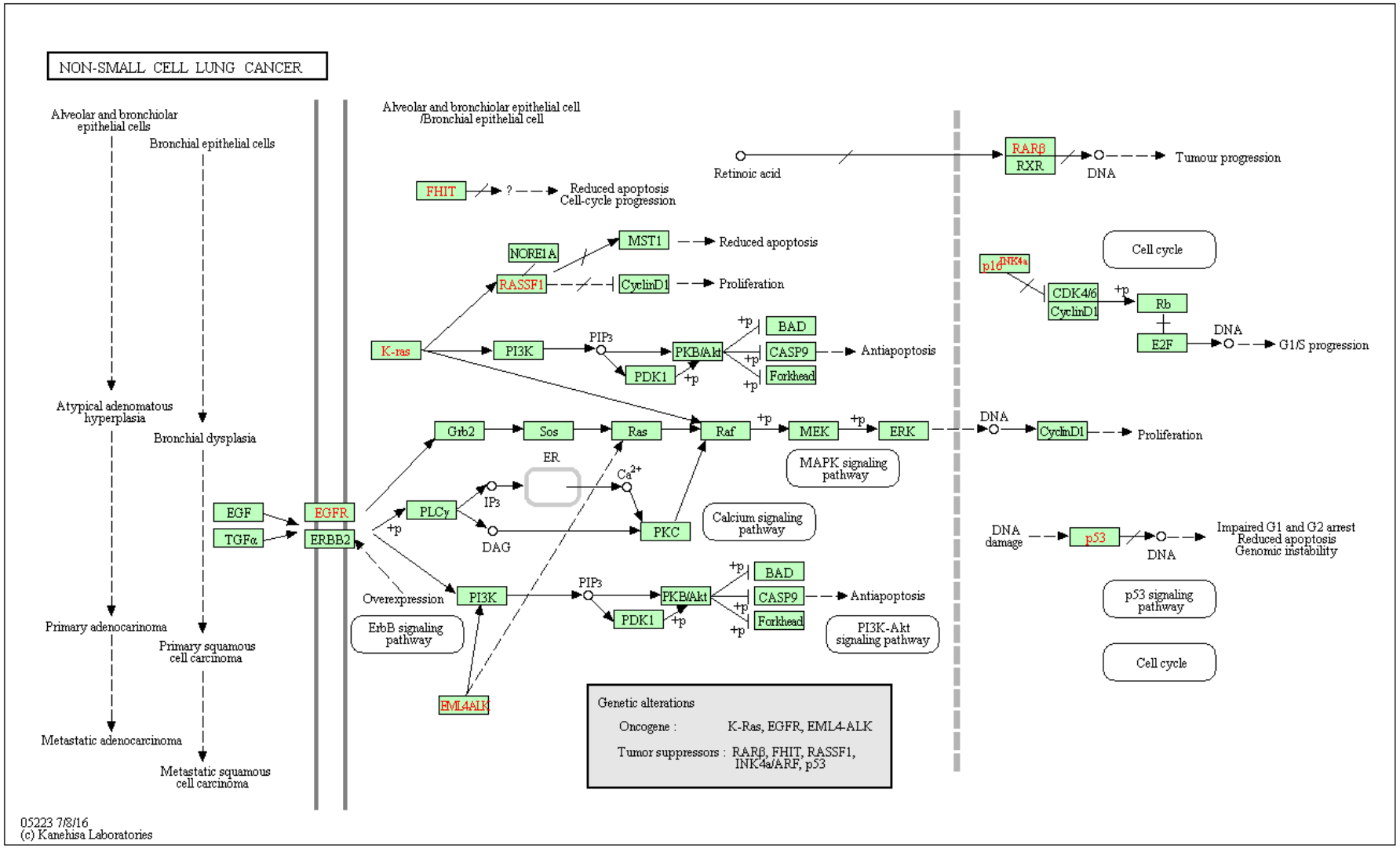
KEGG pathways

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)



Clinical Implications: 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_2174 delTTinsCC (p.Leu725Pro) *GRCh37:* Chr17:37880219-37880220 *GRCh38:* Chr17:39723966-39723967	**ERBB2**	Adenocarcinoma of lung		Pathogenic (Sep 30, 2004)	no assertion criteria provided

Bibliography

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]

http://www.uniprot.org

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

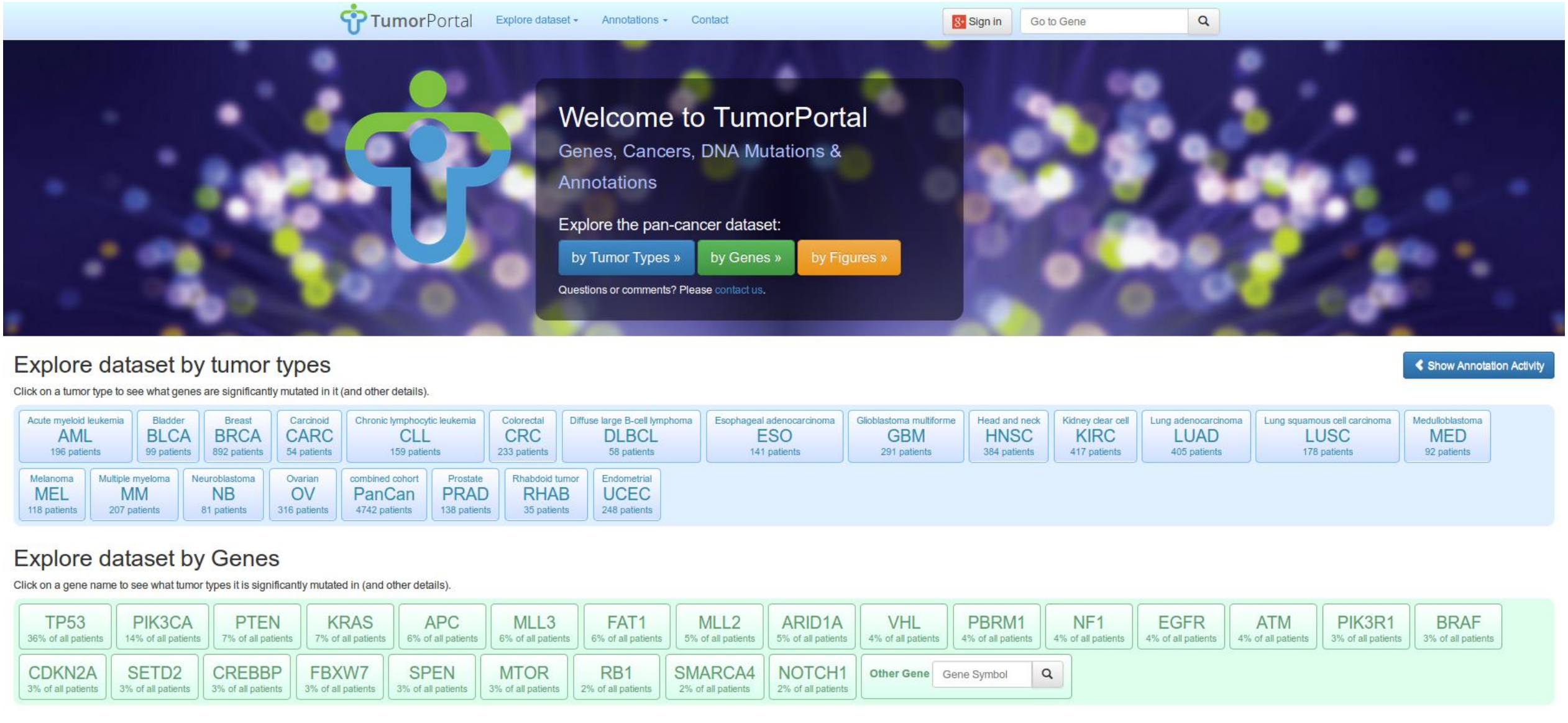
Feature key	Position(s)	Length	Description	Graphical view	Feature identifier	Actions
Disulfide bond[i]	31 ↔ 58					
Glycosylation[i]	56 – 56	1	N-linked (GlcNAc...) (complex); atypical; partia 5 Publications		CAR_000227	
Glycosylation[i]	73 – 73	1	N-linked (GlcNAc...); atypica 1 Publication			
Glycosylation[i]	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			

Specific phenotype information

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

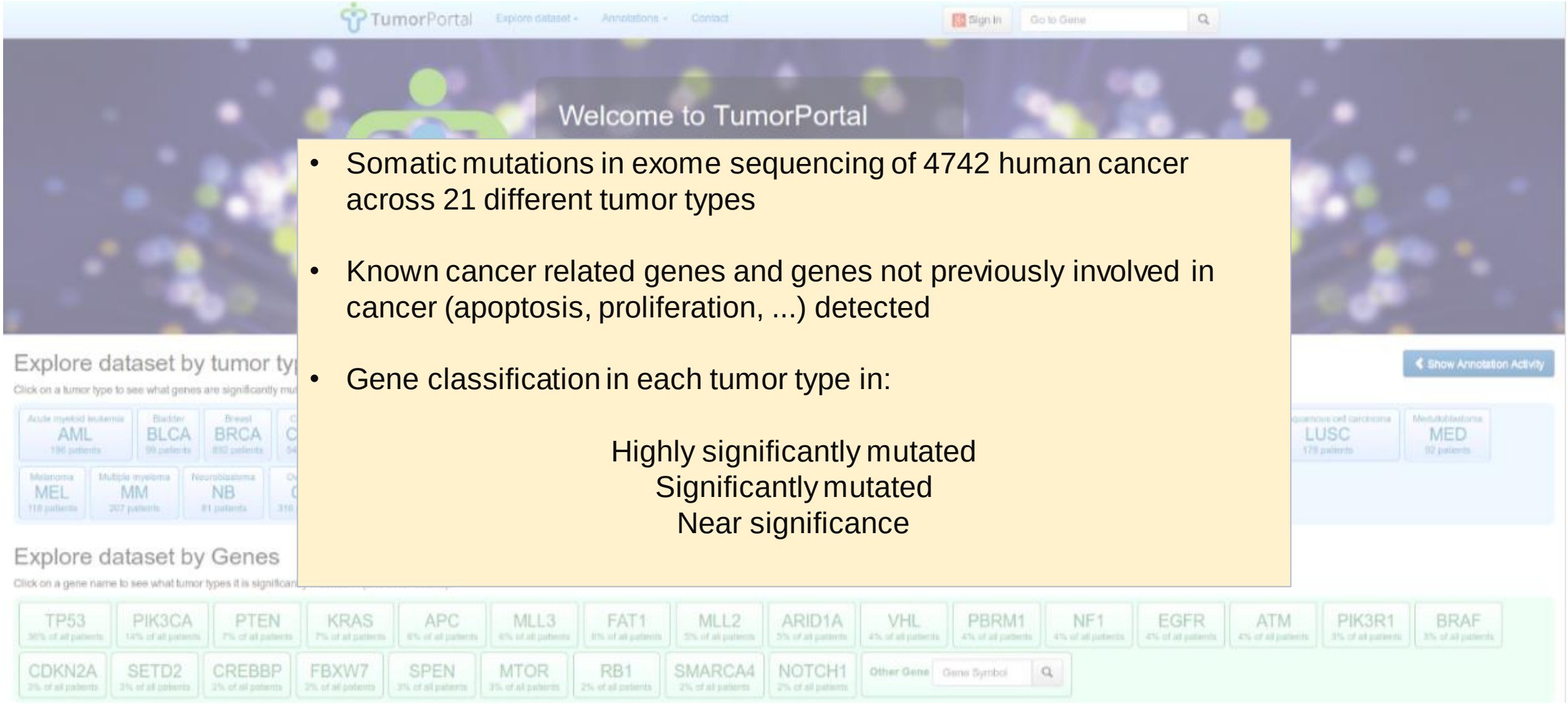
TumorPortal

http://www.tumorportal.org/



TumorPortal

Lawrence et al. Nature 2014



- 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

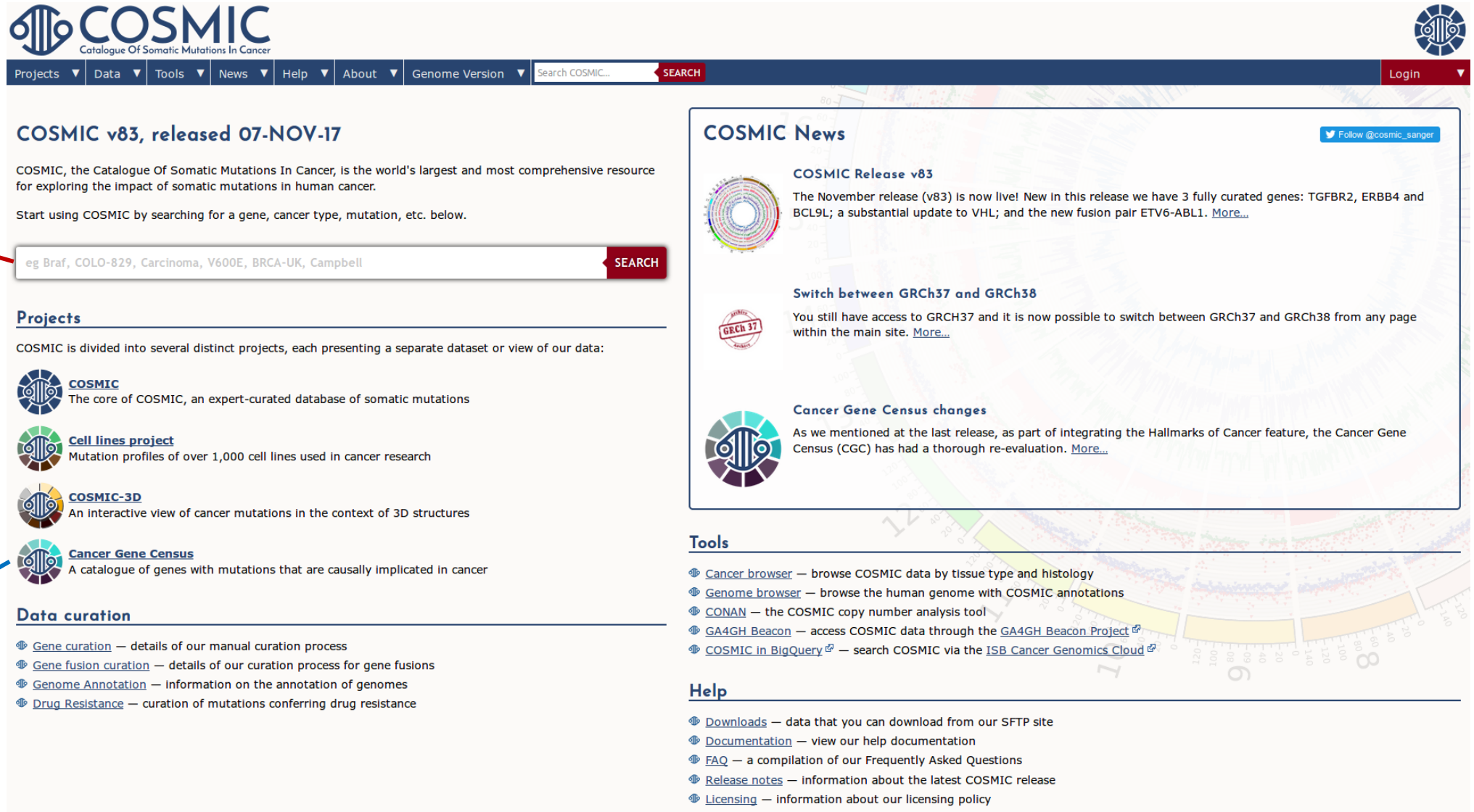
Publication Info:

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.

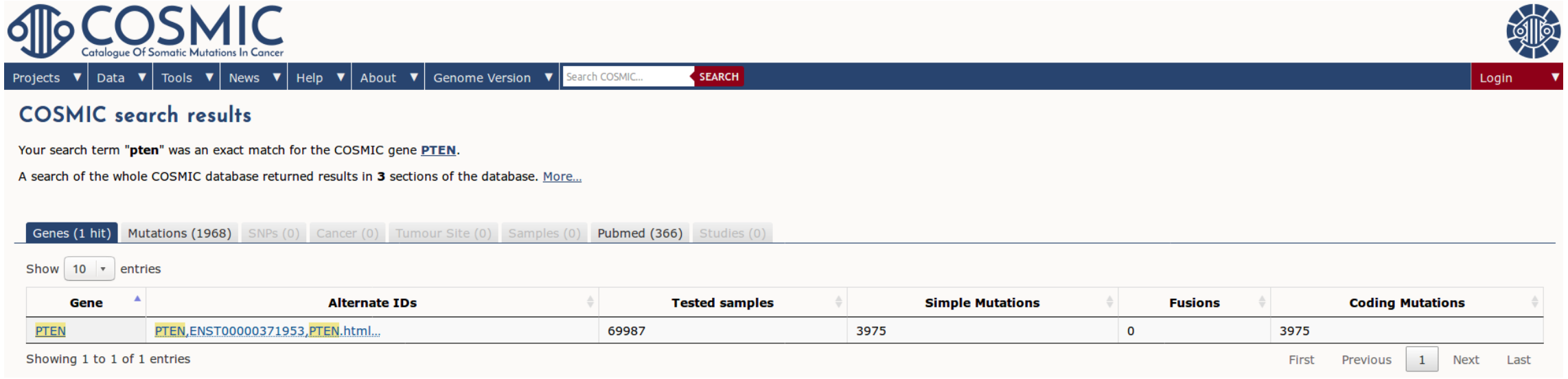
COSMIC additional information

variant/gene frequency in database

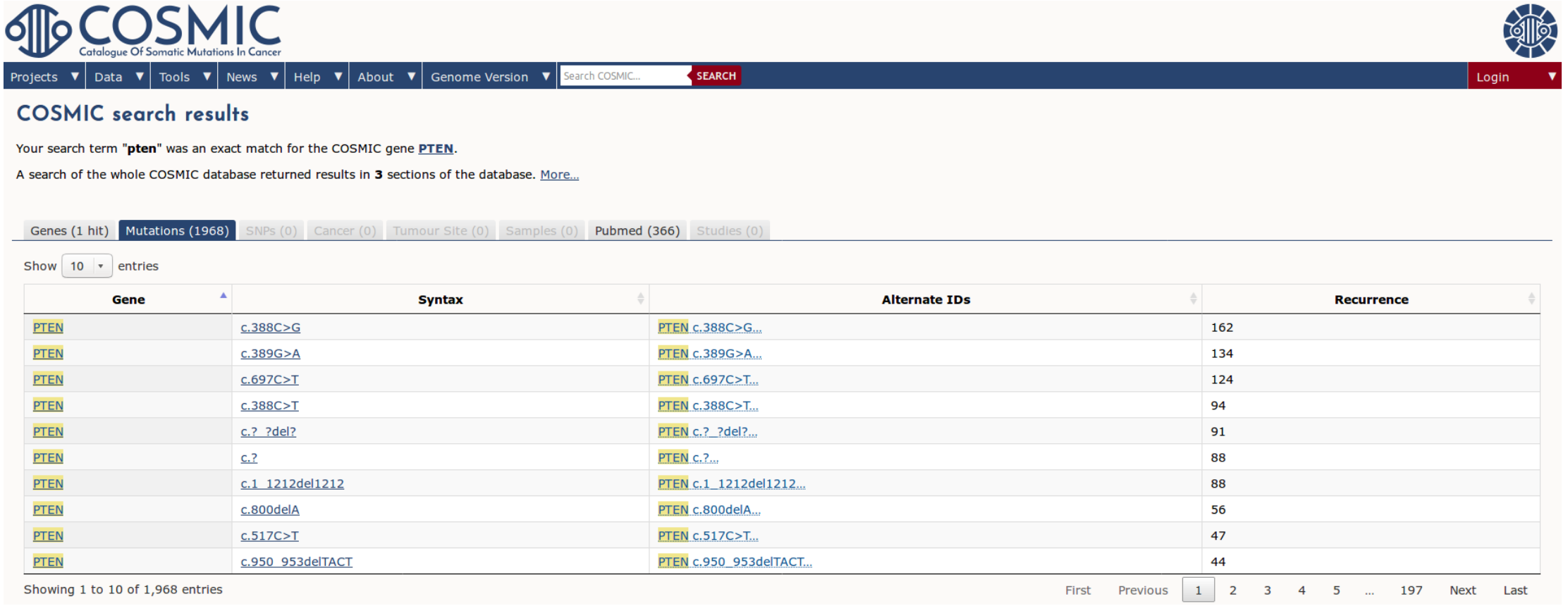
cancer genes information (e.g. role of the gene)



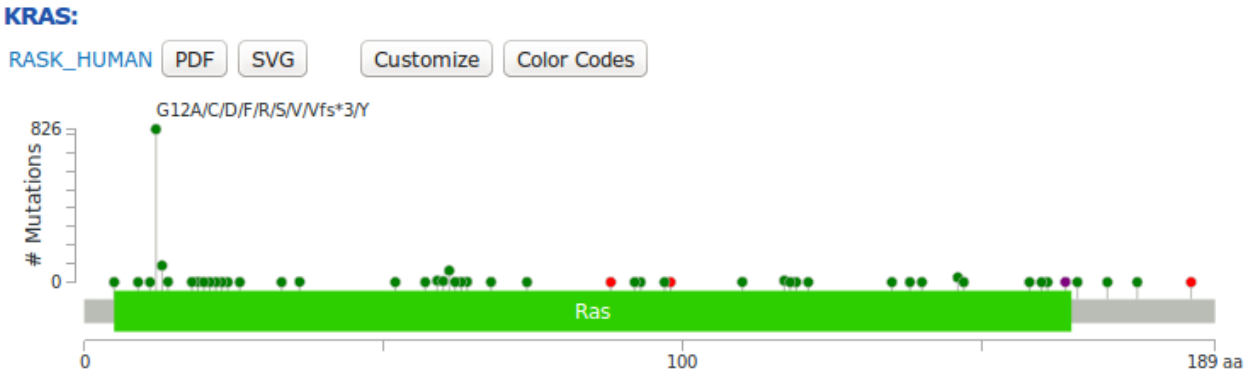
Variant / gene frequency in COSMIC



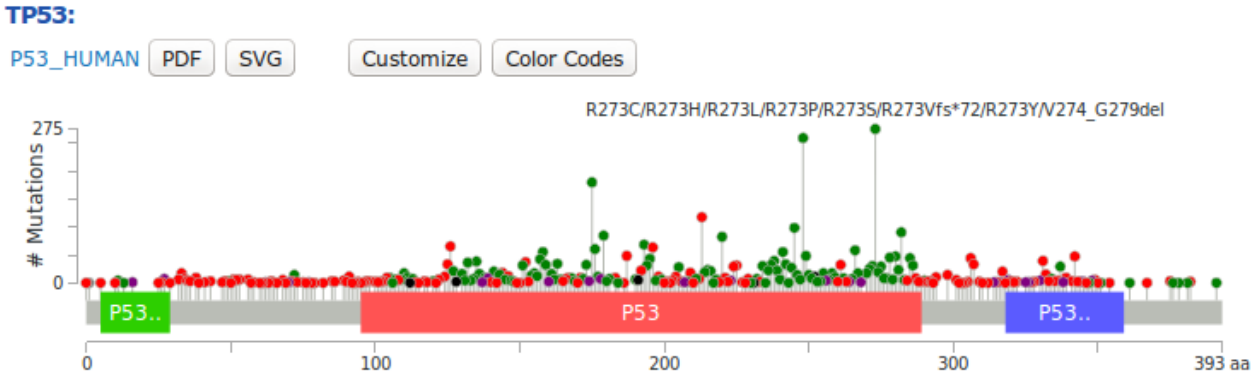
Variant / gene frequency in COSMIC



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/

Cancer Gene Census

Showing both tiers | Show tier 1 | Show tier 2

Show 25 entries Export: CSV TSV Search:

Gene Symbol	Name	Entrez GeneId	Genome Location	Tier	Hallmark	Chr Band	Somatic	Germline	Tumour Types(Somatic)	Tumour Types(Germline)	Cancer Syndrome	Tissue Type	Role in Cancer
ARHGAP5	Rho GTPase activating protein 5	394	14:32090670-32154948	2		14q12	yes		colon cancer; glioma			E; O	oncogene
ARHGEF12	RHO guanine nucleotide exchange factor (GEF) 12 (LARG)	23365	11:120337244-120485077	1		11q23.3	yes		AML			L	TSG; fusion
ARID1A	AT rich interactive domain 1A (SWI-like)	8289	1:26696404-26780756	1		1p35.3	yes		clear cell ovarian carcinoma; RCC; breast			E	TSG
ARID1B	AT rich interactive domain 1B	57492	6:156778104-157207891	1		6q25.1	yes		breast; hepatocellular carcinoma			E	TSG
ARID2	AT rich interactive domain 2	196528	12:45729837-45905078	1		12q12	yes		hepatocellular carcinoma			E	

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

Remove **artifacts**

- Quality filters

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

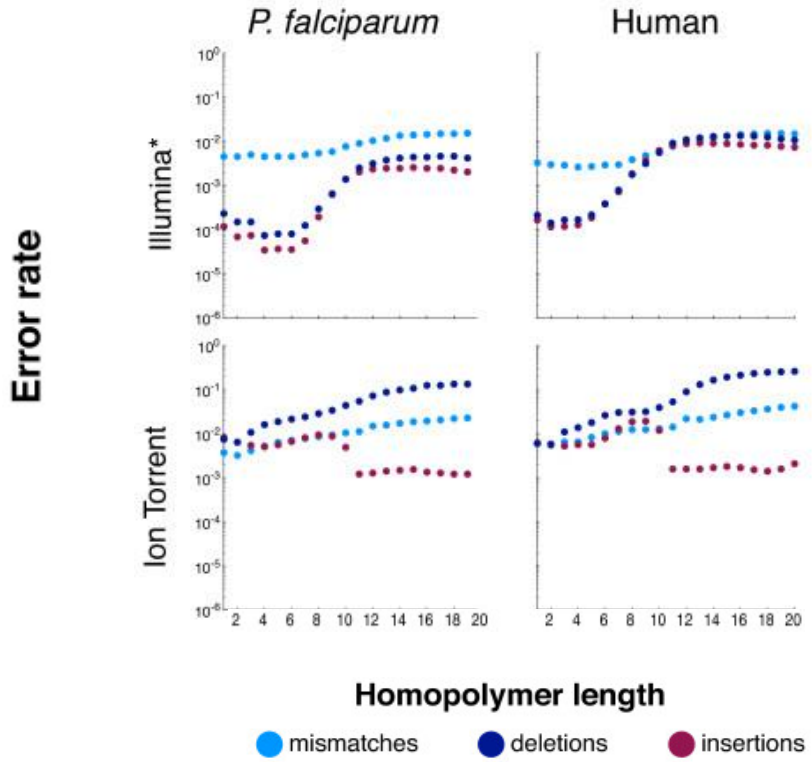
454, 454 pyrosequencing (Roche); NA, not applicable; Nanopore, Oxford Nanopore Technologies; NGS, next-generation sequencing; PacBio, Pacific Biosciences; SOLiD, sequencing by oligonucleotide ligation and detection (Thermo Fisher).

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

Filtering process

Remove **artifacts**

- Quality filters
- Indels in homopolymeric regions
- Repetition in same technology output
  - Repetition can indicate a polymorphism if it is present in at least a 1% of the population
  - Repetition can indicate a frequent alteration if its presence is validated in other samples of the same phenotype
  - Otherwise, it can be an artifact (especially in genes acting as tumor suppressors in cancer)

Filtering process

Remove **artifacts**

- Quality filters
- Indels in homopolymeric regions
- Repetition in same technology output
  - Repetition can indicate a polymorphism if it is present in at least a 1% of the population
  - Repetition can indicate a frequent alteration if its presence is validated in other samples of the same phenotype
  - Otherwise, it can be an artifact (especially in genes acting as tumor suppressors in cancer)
- Variants in positions with very low coverage
- Variants with very low frequency

Filtering process

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

Remove variants affecting **no relevant transcripts**

Remove **polymorphisms** (if not interested in germline information):

Population frequency in 1000 Genomes project, ExAC, gnomAD… >= 1%

Filtering process

We can select 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

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

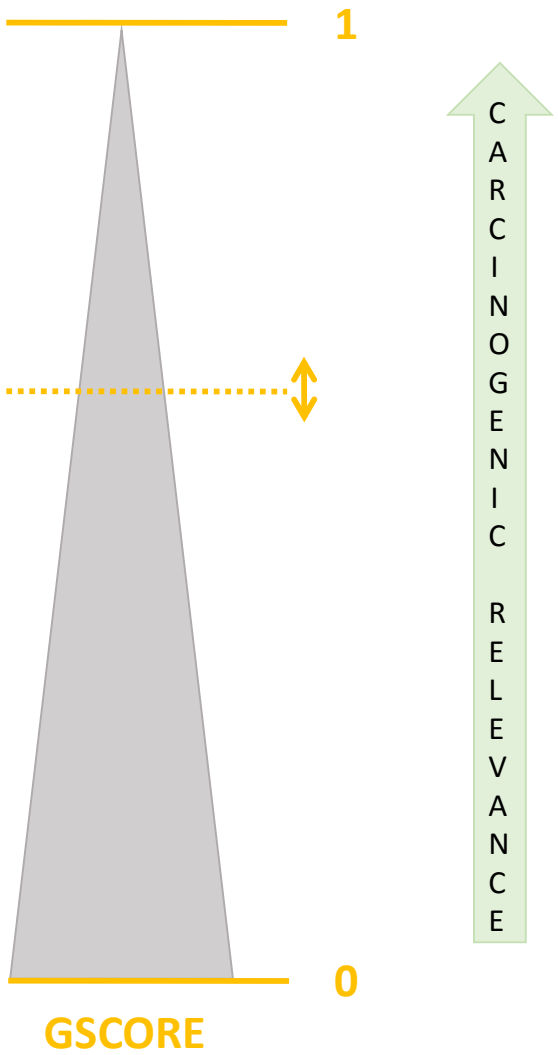
transcript_ablation | splice_donor_variant | splice_acceptor_variant | stop_gained | frameshift_variant | stop_lost | start_lost | transcript_amplification | inframe_insertion | inframe_deletion | missense_variant | protein_altering_variant | splice_region_variant | incomplete_terminal_codon_variant | 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	0.125/3
Score prediction by Sift	<= 0.05	0.125/3	0.125/3
Score prediction by CONDEL	> 0.468	0.125/3	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	0.125
GMAF	< 1	0.125/2	0.125/2
EXAC	< 1	0.125/2	0.125/2
DOMAINS	Listed as relevant in cancer [1] or previous last protein domain (in stop-gained or frameshift)	0.125	0.125
	Within a domain in other circumstances	0.125/2	0.125/2
CLINVAR	Pathogenic with zygosity data	0.125	0.125
	Pathogenic without zygosity data	0.250	0.3125
ZYGOSITY (when available)	Homozygous	0.125	0.1875
ESSENTIALITY SCORE		0.125 * ES	0.125 * ES

Impact

Pathogenicity

Frequencies

Impact

Frequencies

Impact

Pathogenicity

Impact