

Precision medicine: NGS variant analysis and interpretation for translational research

Selecting the most relevant variants: How to filter

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September 28, 2016

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 tumorigenesis	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

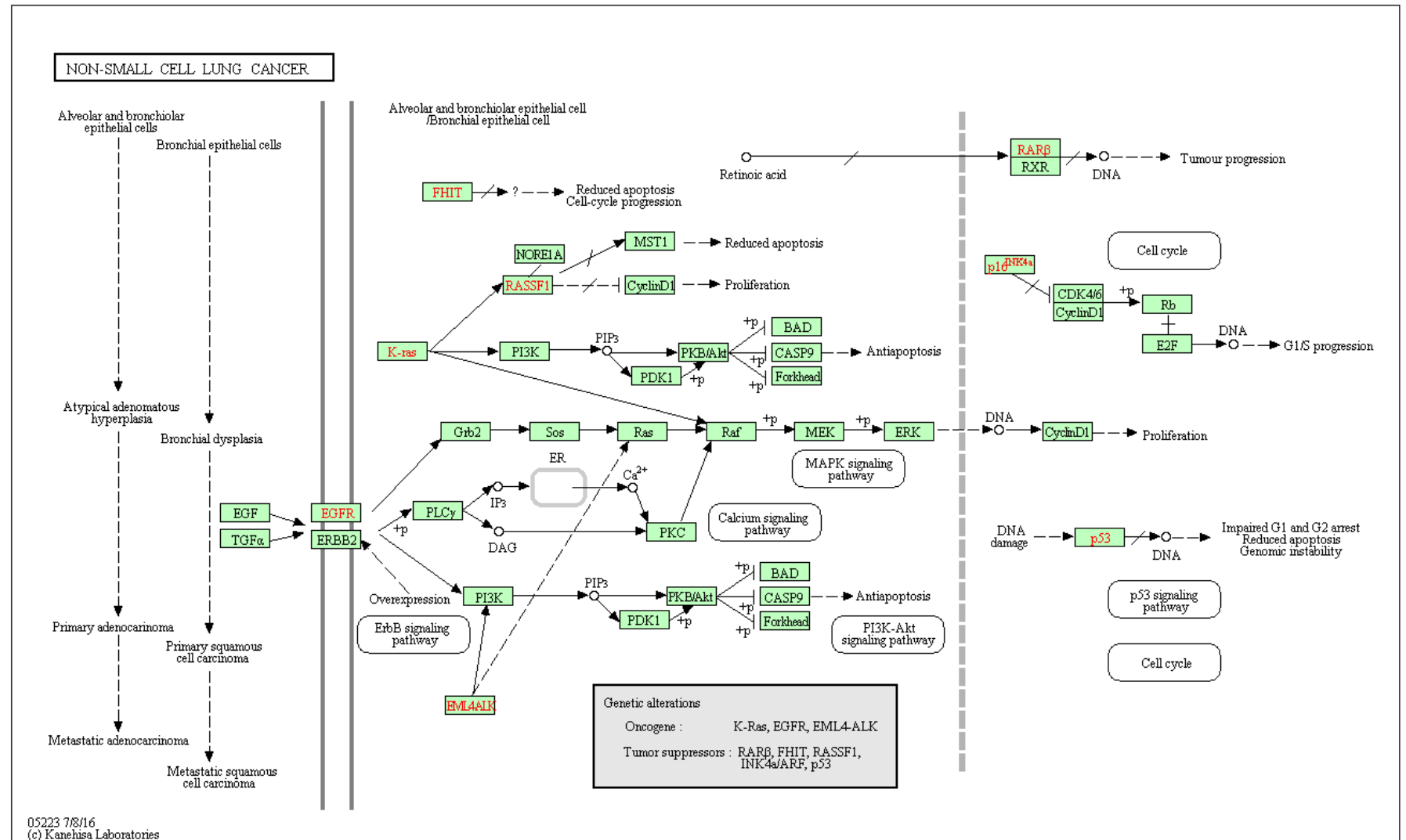
3. Environmental Information Processing

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
<input type="checkbox"/> 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
<input type="checkbox"/> 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
<input type="checkbox"/> 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
<input type="checkbox"/> 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
<input type="checkbox"/> 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

<http://www.uniprot.org>

UniProtKB - P00533 (EGFR_HUMAN)

Protein | **Epidermal growth factor receptor**

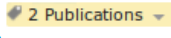






Gene | **EGFR**

Organism | *Homo sapiens (Human)*



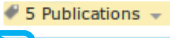

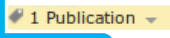

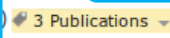
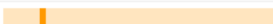

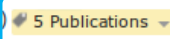
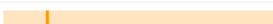


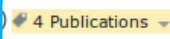
Status |  Reviewed - Annotation score:  - Experimental evidence at protein levelⁱ

PTM / Processingⁱ

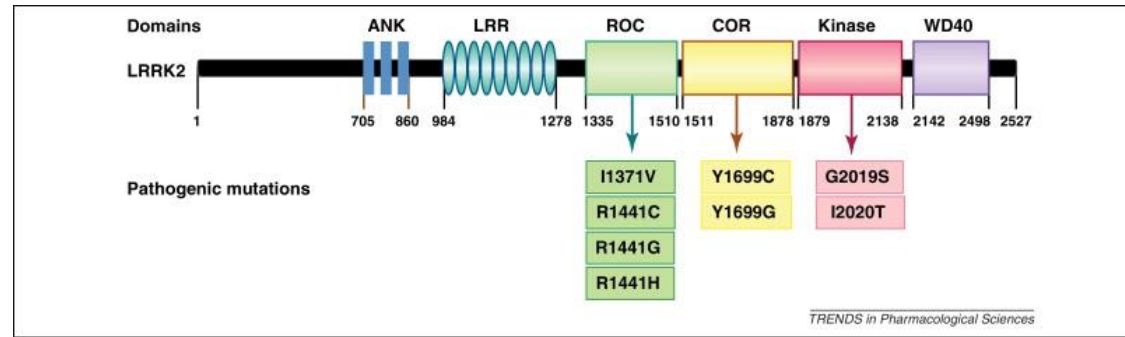
Molecule processing

Feature key	Position(s)	Length	Description	Graphical view	Feature Identifier	Actions
Signal peptide ⁱ	1 – 24	24				 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; partial		CAR_000227	
Glycosylation ⁱ	73 – 73	1	N-linked (GlcNAc...); atypical			
Glycosylation ⁱ	128 – 128	1	N-linked (GlcNAc...)			
Disulfide bond ⁱ	157 ↔ 187					
Glycosylation ⁱ	175 – 175	1	N-linked (GlcNAc...)			
Disulfide bond ⁱ	190 ↔ 199					
Disulfide bond ⁱ	194 ↔ 207					
Glycosylation ⁱ	196 – 196	1	N-linked (GlcNAc...)			

Additional domains information: pfam and Interpro



<https://www.ebi.ac.uk/interpro/>



<http://pfam.xfam.org>

Protein

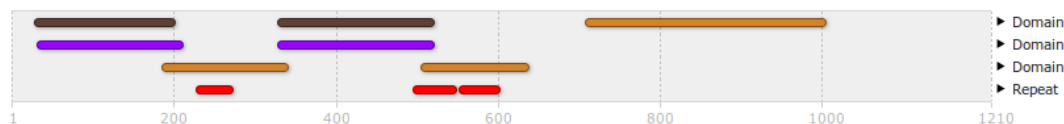
Epidermal growth factor receptor (P00533)

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

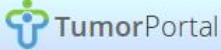
Description:	Epidermal growth factor receptor EC=2.7.10.1
Source organism:	Homo sapiens (Human) (NCBI taxonomy ID 9606) View Pfam proteome data.
Length:	1210 amino acids
Reference Proteome:	✓

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

Explore dataset ▾Annotations ▾Contact

Sign inGo to Gene



Welcome to TumorPortal

Genes, Cancers, DNA Mutations & Annotations

Explore the pan-cancer dataset:

by Tumor Types »by Genes »by Figures »

Questions or comments? Please [contact us](#).

Explore dataset by tumor types

◀ Show Annotation Activity

Click on a tumor type to see what genes are significantly mutated in it (and other details).

Acute myeloid leukemia AML 196 patients	Bladder BLCA 99 patients	Breast BRCA 892 patients	Carcinoid CARC 54 patients	Chronic lymphocytic leukemia CLL 159 patients	Colorectal CRC 233 patients	Diffuse large B-cell lymphoma DLBCL 58 patients	Esophageal adenocarcinoma ESO 141 patients	Glioblastoma multiforme GBM 291 patients	Head and neck HNSC 384 patients	Kidney clear cell KIRC 417 patients	Lung adenocarcinoma LUAD 405 patients	Lung squamous cell carcinoma LUSC 178 patients	Medulloblastoma MED 92 patients
Melanoma MEL 118 patients	Multiple myeloma MM 207 patients	Neuroblastoma NB 81 patients	Ovarian OV 316 patients	combined cohort PanCan 4742 patients	Prostate PRAD 138 patients	Rhabdoid tumor RHAB 35 patients	Endometrial UCEC 248 patients						

Explore dataset by Genes

Click on a gene name to see what tumor types it is significantly mutated in (and other details).

TP53 36% of all patients	PIK3CA 14% of all patients	PTEN 7% of all patients	KRAS 7% of all patients	APC 6% of all patients	MLL3 6% of all patients	FAT1 6% of all patients	MLL2 5% of all patients	ARID1A 5% of all patients	VHL 4% of all patients	PBRM1 4% of all patients	NF1 4% of all patients	EGFR 4% of all patients	ATM 4% of all patients	PIK3R1 3% of all patients	BRAF 3% of all patients
CDKN2A 3% of all patients	SETD2 3% of all patients	CREBBP 3% of all patients	FBXW7 3% of all patients	SPEN 3% of all patients	MTOR 3% of all patients	RB1 2% of all patients	SMARCA4 2% of all patients	NOTCH1 2% of all patients	Other Gene	Gene Symbol					

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

The screenshot displays the TumorPortal website. At the top, the header includes the TumorPortal logo, navigation links for 'Explore dataset', 'Annotations', and 'Contact', a 'Sign In' button, and a 'Go to Gene' search bar. A large banner area features a 'Welcome to TumorPortal' message. Below this, the 'Explore dataset by tumor type' section is visible, with a grid of buttons for various cancer types: AML (196 patients), BLCA (59 patients), BRCA (892 patients), MEL (118 patients), MM (207 patients), NB (81 patients), LUSC (179 patients), and MED (92 patients). A 'Show Annotation Activity' button is also present. The 'Explore dataset by Genes' section at the bottom shows a grid of gene buttons, each indicating the percentage of patients with a mutation: TP53 (36%), PIK3CA (14%), PTEN (7%), KRAS (7%), APC (6%), MLL3 (6%), FAT1 (6%), MLL2 (5%), ARID1A (5%), VHL (4%), PBRM1 (4%), NF1 (4%), EGFR (4%), ATM (4%), PIK3R1 (3%), BRAF (3%), CDKN2A (2%), SETD2 (2%), CREBBP (2%), FBXW7 (2%), SPEN (3%), MTOR (3%), RB1 (2%), SMARCA4 (2%), and NOTCH1 (2%). An 'Other Gene' search bar is located at the bottom right of the gene grid.

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: ccl-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



[Home](#) [About](#) [Resources](#) [Curation](#) [Tools](#) [Data](#) [News](#) [Help](#) [Login](#)

COSMIC v77

[SEARCH](#)

R Resources

Key COSMIC resources

- [Cell Lines Project](#)
- [COSMIC Whole Genomes](#)
- [Cancer Gene Census](#)
- [Drug Sensitivity](#)
- [Mutational Signatures](#)
- [GRCh37 Cancer Archive](#)

T Tools

Additional tools to explore COSMIC

- [Cancer Browser](#)
- [Genome Browser](#)
- [GA4GH Beacon](#)
- [COSMIC Mart](#)
- [CONAN](#)

C Expert Curation

High quality curation by expert postdoctoral scientists

- [Drug Resistance^{New}](#)
- [Cancer Gene Census](#)
- [Curated Genes](#)
- [Gene Fusions](#)
- [Genome-Wide Screens](#)

D Data

Further details on using COSMIC's content

- [Downloads](#)
- [License](#)
- [Submission](#)
- [Genome Annotation](#)
- [Datasheets](#)
- [Help](#)
- [FAQ](#)

Genomic Landscape of Cancer

Statistics

Domain	Counts
Samples	1209567
Coding Mutations	4118156
Papers	23084
Fusions	17628
Whole Genomes	25875
Copy Number	1064039
Gene Expression	9479893
Differentially Methylated CpGs	7879142

variant/gene frequency
in database

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

Variant / gene frequency in COSMIC



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COSMIC search results

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

Show entries Search:

Keyword search: [Go](#)

Type ⁱ	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 entries

Gene	Alt Ids	Tested samples	Simple Mutations	Fusions	Coding Mutations
PTEN	PTEN...	32968	754	0	754

Showing 1 to 1 of 1 entries [First](#) [Previous](#) [1](#) [Next](#) [Last](#)

Variant / gene frequency in COSMIC



COSMIC search results

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

Show entries Search:

Type ⁱ	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 Next

Keyword search:

Go

Genes Mutations Pubmed

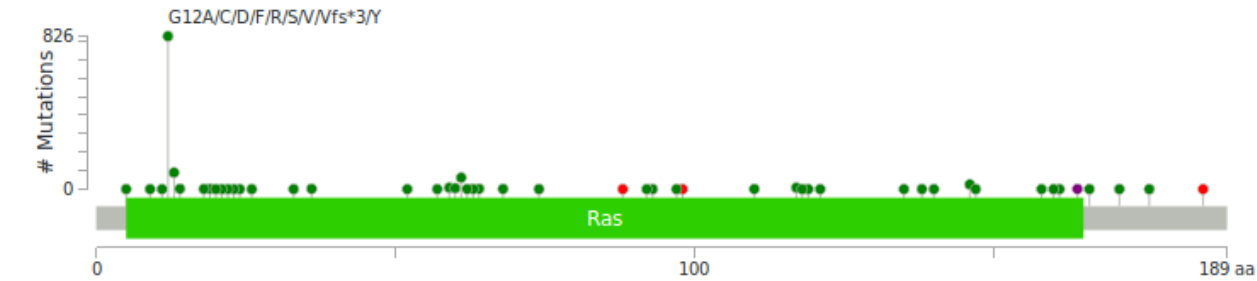
Show entries

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

KRAS:

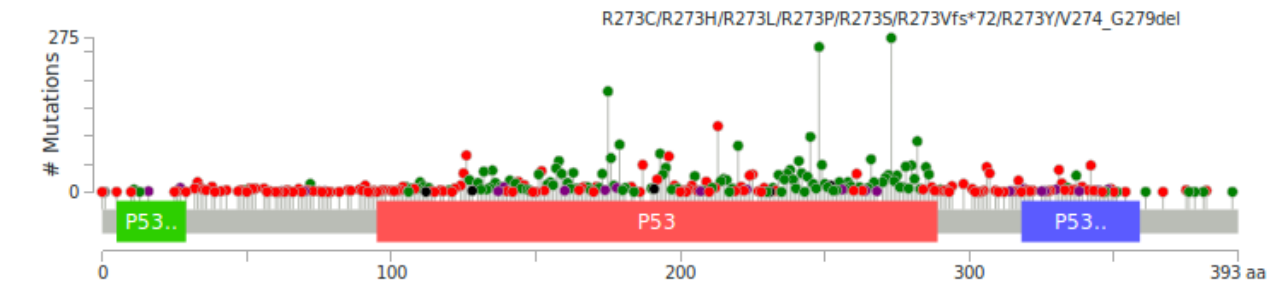
RASK_HUMAN PDF SVG Customize Color Codes



ONCOGENE

TP53:

P53_HUMAN PDF SVG Customize Color Codes



TUMOR SUPPRESSOR GENE

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

Role of the gene as ONC or TSG



<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
ENSG000000009307	CSDE1	Loss of function	0.2515
ENSG000000012048	BRCA1	Loss of function	0.0020
ENSG000000019991	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 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

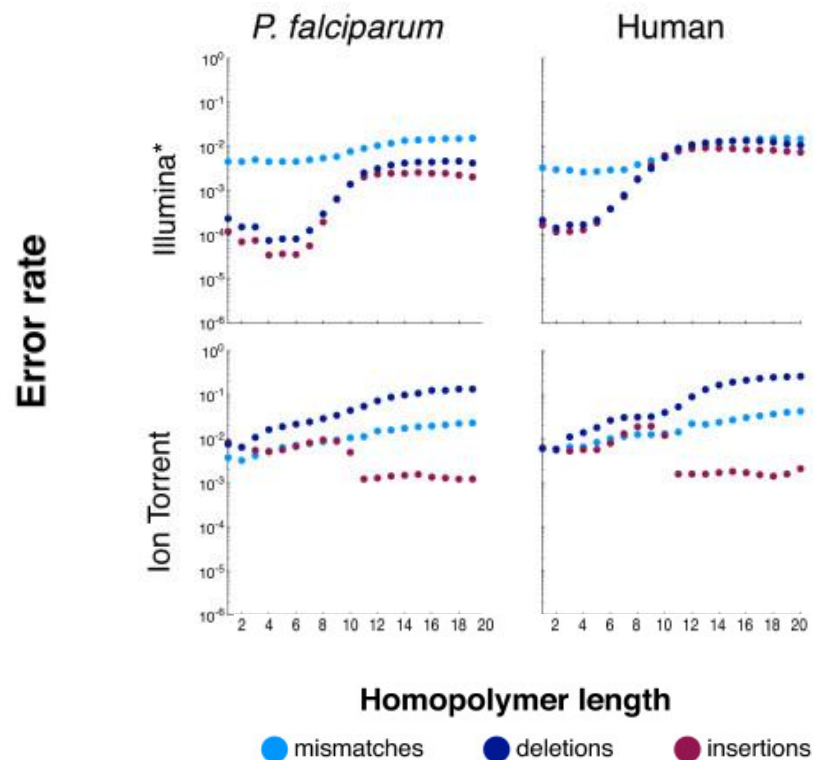
454, 454 pyrosequencing (Roche); NA, not applicable; Nanopore, Oxford Nanopore Technologies; NGS, next-generation sequencing; PacBio, Pacific Biosciences; SOLiD, sequencing by oligonucleotide ligation and detection (Thermo Fisher).

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

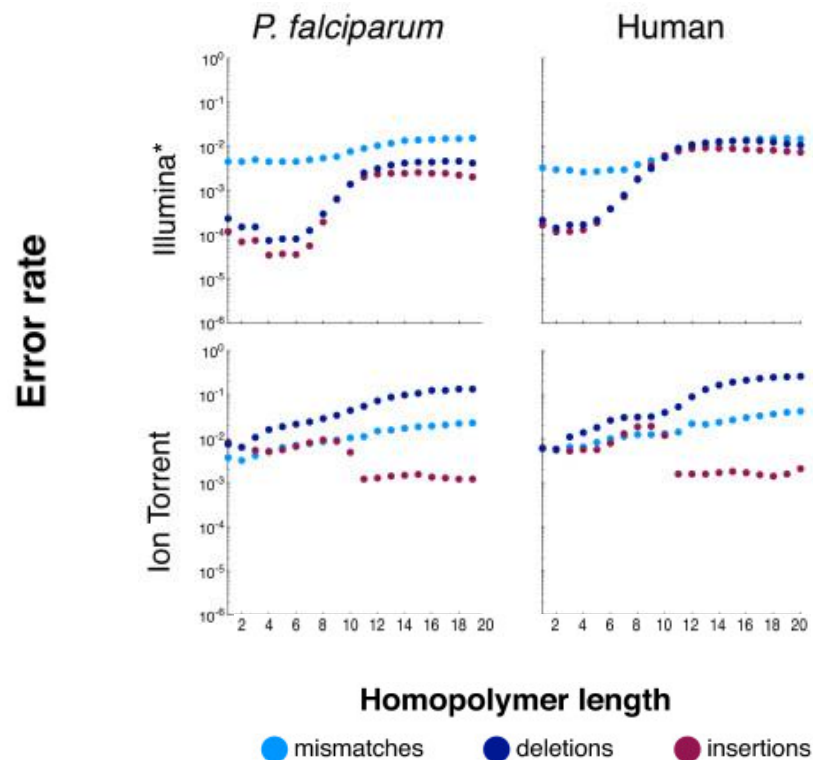
- Base-calling errors
 - Indel errors: Rare in Illumina. Main source of errors in IonTorrent and 454.
 - Substitution errors: Dominant in Illumina and SOLiD platforms.



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.



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)

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, ... $\geq 1\%$ (if not interested in germline information)

Filtering criteria

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

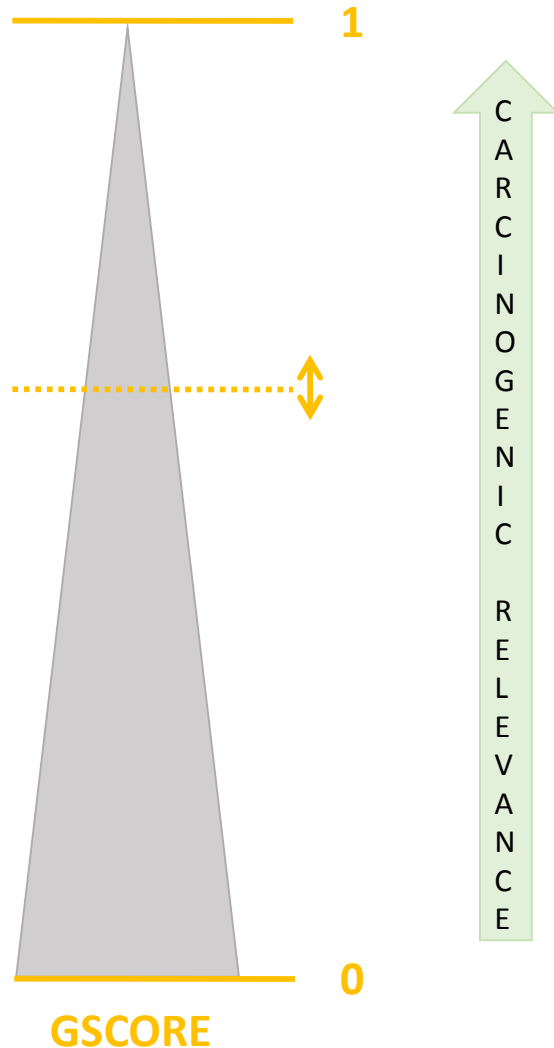
transcript_ablation | splice_donor_variant | splice_acceptor_variant | stop_gained |
frameshift_variant | stop_lost | start_lost | transcript_amplification | inframe_insertion |
inframe_deletion | missense_variant | protein_altering_variant | splice_region_variant |
incomplete_terminal_codon_variant | 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	$(0.125 / 3) * (\log(\text{mutation frequency}) / \log(\text{maximum mutation frequency}))$	
Frequency of gene in COSMIC	>= 100	0.125/3	0.03125
	< 100	$(0.125 / 3) * (\log(\text{gene frequency}) / \log(\text{maximum gene frequency}))$	$0.03125 * (\log(\text{gene frequency}) / \log(\text{maximum gene frequency}))$
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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