

Help



Submit variants for analysis

Example

Tutorial


Resources

References

Annotation Resources

- dbNSFP
- PhosphoELM
- FireDB
- SNP2L
- iHOP
- STRING
- Structure-PPI
- Uniprot
- Kin-Driver
- KinMutBase
- COSMIC
- ClinVar

Kinase variants
(e.g. P150S6 V600E)



Kinase group
Gene essentiality
US FDA approved drugs
Pathogenicity predictions
Functional/Catalytic residues
Interaction interfaces and partners
Database and Literature mining

Prediction Resources

- KinMutRF
- MutationTaster
- Polyphen2-hdiv
- Polyphen2-hvar
- LRT
- SIFT
- MutationAssessor
- FATHMM
- VEST3
- CADD

wKinMut-2 is an integrated framework for the analysis and interpretation of the consequences of variants in the human kinome.

It displays information from diverse sources, that include:

- Protein/Gene general information, Gene Ontology terms, estimations of gene essentiality in orthologues and known drug targets.
- Structural information from PDB and Structure-PPI.
- Characterization at the domain and residue levels. Functional annotations and information about plausible catalytic and phosphorylation sites.
- Predictions of variant pathogenicity with 9 different methods, including our own kinase-specific Random Forest.
- Information from dedicated variant databases (UniProt Variant Pages, COSMIC, KinMutBase, Kin-Driver, ClinVar)
- Variants mentions from the literature (SNP2L)
- Protein Protein Interactions extracted from the literature (iHOP) and STRING.

Kinmut2.bioinfo.cnio.es presents a welcome home page that includes link to useful examples, tutorials, a summary of the resources and the references to our KinMut related publications. To begin the analysis of variants, please, click on “Submit variants for analysis” (an orange arrow will identify clickable areas in this tutorial).

The screenshot shows the wKinMut2 web interface. At the top left is the logo 'wKinMut2' in red and black. At the top right is a 'Help' button. Below the logo is a large text input area for 'Variants'. To the right of this area is explanatory text. At the bottom are three buttons: 'Try example', 'Manuscript', and 'Submit'. Three orange arrows with numbers 1, 2, and 3 point to the variant input area, the 'Manuscript' button, and the 'Submit' button respectively.

Variants


P15056 V600E
P07949 A883F

The input to the system are single point missense events affecting the human kinome. Variants are defined by a Uniprot accession, a position in the protein and the wild-type and alternative amino acids. Consequently, a change from Valine to Glutamate in position 600 of the B-Raf proto-oncogene would be encoded as P15056 V600E.

Non-standard amino acids will be decomposed into separate instances of their standard counterparts (D, N and E, Q, respectively) whereas synonymous and truncating variants will be excluded from the analysis. Additionally, we highlight in red input instances where the introduced wild-type amino acid does not coincide with the expected equivalent position in the canonical protein sequence.

Try example Manuscript Submit

- (1) The input to wKinMut-2 are single point missense variants affecting human protein kinases. A variant can be defined in a very simple format using the accession number from UniProt, the wild-type and mutant amino acids and the position in the protein. For example, the well studied change from Valine (V) to Glutamate (E) in position 600 of the B-Raf proto-oncogene (UniProt accession number: P15056) would be encoded as “P15056 V600E”. Multiple variants can be analysed at a time using wKinMut-2, for that the input should contain one variant per line.
Please, note that the server displays information for an alternative amino acid at a time. Non-standard amino acids (B,Z) will be decomposed into separate instances of their standard counterparts. For example, “P07949 A883B” would be read internally as “P07949 A883D” and “P07949 A883N”. wKinMut-2 focuses on the analysis of missense variants. Synonymous and truncating variants will be excluded from the analysis.
- (2) After introducing the variant, user can optionally name the experiment.
- (3) Press the “Submit” button to start running the analysis. Depending on the load of the server and the number of variants submitted for analysis, some calculations might take some time to be ready. Please, be patient.


Help

Job: [Manuscript](#)

The following results are the *KinMut2*-RandomForest predictions for the potential disease implications of each mutation. You can view each in more detail by clicking the buttons on each row.

The results are available for download below the table, as well as the correspondence between the input protein identifiers used as input and their correspondent UniProt accessions, the features used by the algorithm, and it's raw input and output. Additionally we offer some of the in-depth annotations also available for bulk download: Appris, COSMIC, UniProt, dbNSFP, PPI interfaces, etc

KG: Classification of the human kinome according to Manning et al., 2002

#CS: Number of COSMIC samples with variants overlapping that same residue. The number of samples with mutations close to the variant is shown in parenthesis

MR: Variant overlapping a post-translational modifications (UniProt feature: MOD_RES) annotated residue. The number of such residues close to the variant is shown in parenthesis

DT: The protein is targeted by an FDA approved drug

ODP: Number of other damage predictors that predict the mutation as damaging: SIFT, Polyphen2_HDIV, Polyphen2_HVAR, MutationTaster, MutationAssessor, FATHMM, VEST3, and CADD

KM Score: KinMut-RF prediction score. The closer to 1 in absolute value the higher the confidence

CV: Marked pathogenic in ClinVar

FL: Variant overlapping a Firestar ligand binding or catalytic residue. The number of Firestar annotated residues close to the variant is shown in parenthesis

MUT: Variant overlapping a residue experimentally altered by mutagenesis (UniProt feature: MUTAGEN). The number of such residues close to the variant is shown in parenthesis

PPI: The mutation affects a protein-protein interaction interface

KM Pred.: KinMut-RF prediction

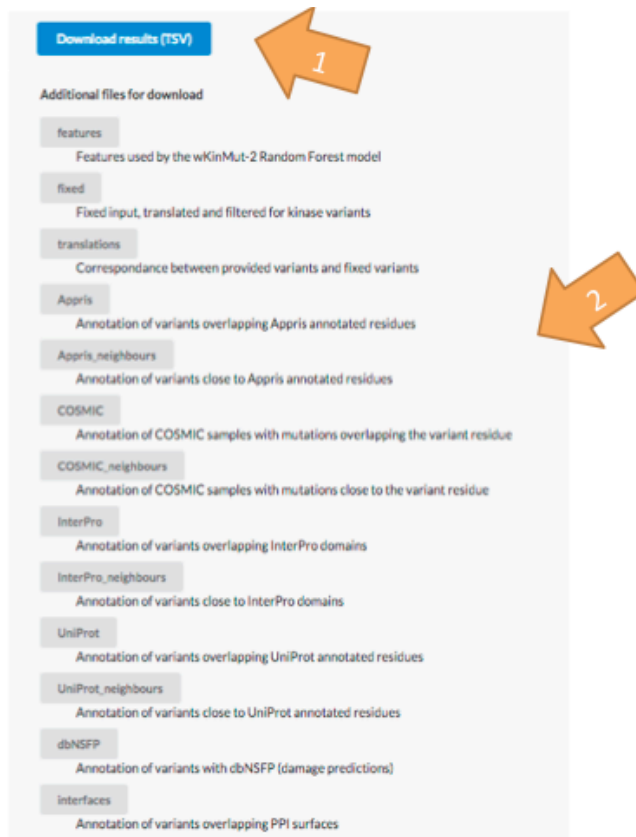
| Kinase | Variant | KG | CV | #CS | FL | MR | MUT | DT | PPI | ODP | KM Pred. | KM Score | |
|---------------|---------|-----|-----|--------|--------|--------|----------|-----|-----|--------|----------|----------|------------------------------|
| P07949 (RET) | A883F | Tyr | No | 3 (2) | No (4) | No (2) | Yes (12) | Yes | No | NA | disease | 0.846 | View details |
| P15056 (BRAF) | V600E | TKL | Yes | 51 (0) | No (0) | No (0) | No (0) | Yes | Yes | 6 of 8 | disease | 0.669 | View details |

* Kinase Group: Classification of the human kinome according to Manning et al., 2002.

- TKL: tyrosine protein kinase-like
- Tyr: tyrosine protein kinase

[Download results \(TSV\)](#)

- (1) After the calculation for the submitted variants has finished, the user is presented with a table that summarizes these results. The table includes information to start the prioritization of mutations for further analysis including: KG: The classification of the kinase in the context of the human kinome according to Manning's taxonomy; CV: The mutation has been classified as pathogenic in ClinVar; #CS: The number of COSMIC (somatic mutations in cancer) samples with variants in the same residue; FL: The variant sits on a residue that Firestar considers relevant for ligand binding; MR: Whether the residue is annotated as subject of translational modification (MOD_RES) in UniProt; MUT: Whether the residue is annotated as subject to mutagenesis (MUTAGEN) in UniProt, under the assumption that targeted experiments would focus on functionally relevant residues. The number in parenthesis reflects the amount of residues in the proximity that fulfill these criteria; DT: Whether the protein is targeted by a FDA approved drug; PPI: The residue is involved in a protein-protein interface; ODP: wKinMut-2 calculates the consequences of variants according to 8 external classifiers: SIFT, Polyphen2-HDIV, Polyphen2-HVAR, MutationTaster, MutationAssessor, FATHMM, VEST, and CADD. The ODP (Other Disease Predictors) reflects how many of these classify the variant as pathogenic; "KM Pred." and "KM Score": In addition, the variants are evaluated with a random forest (KinMutRF) developed ad-hoc for the study of human protein kinases. Variants are classified disease or neutral and a reliability index (ranging from 0 to 1) will assess the confidence in the prediction. The closer to 1 in absolute value the higher the confidence.
- (2) Once the variants of interest have been identified, users can obtain additional information by clicking on "View details" button.



- (1) Some users might find themselves interested in having a report of the predictions. These can be downloaded directly from the server as a tab-separated (TSV) file.
- (2) Additional files used for the prediction or containing supplemental information can be downloaded as well.

Details: Manuscript > (BRAF) P15056 - V600E

General Structures Structure-PPI Pathogenicity Databases Literature iHOP String

Gene Name
BRAF
essential gene (from dbNSFP)

UniProt accession
P15056

Ensembl protein identifier
ENSP00000288602

Long Name
B-Raf proto-oncogene, serine/threonine kinase

Summary
This gene encodes a protein belonging to the raf/mil family of serine/threonine protein kinases. This protein plays a role in regulating the MAP kinase/ERKs signaling pathway, which affects cell division, differentiation, and secretion. Mutations in this gene are associated with cardiofaciocutaneous syndrome, a disease characterized by heart defects, mental retardation and a distinctive facial appearance. Mutations in this gene have also been associated with various cancers, including non-Hodgkin lymphoma, colorectal cancer, malignant melanoma, thyroid carcinoma, non-small cell lung carcinoma, and adenocarcinoma of lung. A pseudogene, which is located on chromosome X, has been identified for this gene.

Kinase Group
TKL: tyrosine protein kinase-like. (Log odds ratio: 1.910551)

FDA approved inhibitors

| Drug | Sponsor | Indications | Target |
|-------------|-------------------|--|---|
| Regorafenib | Bayer | Advanced colorectal cancer, gastrointestinal stromal tumours | RET, VEGFR, PDGFR, Bcr-Abl, BRAF, (4 more) |
| Sorafenib | Bayer | Advanced Renal cell carcinoma and Hepatocellular carcinoma | VEGFR, PDGFR, BRAF, CRAF, c-KIT, FLT3, RET. |
| Vemurafenib | Hoffmann-La Roche | Metastatic malignant melanoma | ARAF, BRAF, CRAF |
| Dabrafenib | GlaxoSmithKline | Melanoma | BRAF |

GO terms

Biological Process Molecular Function Cellular component

| Gene Ontology - Biological Process | Log-odds ratio |
|---|----------------|
| GO:0071277 cellular response to calcium ion | 4.5557 |
| GO:0051291 protein heterooligomerization | 3.56 |
| GO:0043368 positive T cell selection | 3.5013 |

- (1) The general tab will describe basic features of the kinase where the variant of interest occurred.
- (2) Information present includes the gene name and the description from UniProt, the protein identifier in Ensembl and the classification in kinase groups as defined by Manning and collaborators. In addition, as a proxy to understand the cellular role of the protein, we list GeneOntology annotations grouped by sub-ontology (i.e., Molecular Function, Cellular Compartment and Biological Process).
- (3) And we provide information about:
 - i) Essential or non-essential phenotype-changing of the homologous gene in mouse based on the information collected by dbNSFP [Liu et al. 2013] from the Mouse Genome Informatics database (<https://sites.google.com/site/jpopgen/dbNSFP>).
 - ii) US FDA [Jänne et al. 2009] approved protein kinase inhibitors (<http://www.brimr.org/PKI/PKIs.htm>).



- (1) The Structure tab represents the variants with respect to the protein structures of the kinases.
- (2) A Jmol representation of the structures and the variants helps understand the functional consequences of the latter.
- (3) Domain information is also provided to elucidate the potential impact of the variants on the function of the kinases.

Details: Manuscript > (BRAF)P15056-V600E

General Structures Structure-PPI Pathogenicity Databases Literature iHOP String

Affected PPI interface

| Partner protein | Close partner residues |
|-----------------|------------------------|
| PAK2 | 287, 288, 289 |
| BRAF | 448 |

COSMIC

| Genomic Mutation | Sample ID | Primary site | Site subtype | Primary histology | Histology subtype | PMID |
|------------------|-----------|--------------|--------------|--------------------------|---------------------|----------|
| 7:140453137:A | 1024063 | prostate | NS | carcinoma | adenocarcinoma | 16721785 |
| 7:140453136:C | 1024064 | prostate | NS | carcinoma | adenocarcinoma | 16721785 |
| 7:140453136:C | 1024066 | prostate | NS | carcinoma | adenocarcinoma | 16721785 |
| 7:140453136:G | 1024067 | prostate | NS | carcinoma | adenocarcinoma | 16721785 |
| 7:140453137:T | 1024071 | prostate | NS | carcinoma | adenocarcinoma | 16721785 |
| 46 more | 46 more | 46 more | 46 more | 46 more | 46 more | 46 more |
| Neighbour 601 | | | | | | |
| 7:140453134:G | 1045129 | skin | NS | benign_melanocytic_nevus | Spitz | 16987295 |
| 7:140453133:G | 1112823 | skin | NS | malignant_melanoma | NS | 17824790 |
| 7:140453132:G | 1220509 | thyroid | NS | carcinoma | papillary_carcinoma | 17785355 |

- (1) Structure-PPI is a system to facilitate the analysis of variation in the context of protein complexes.
- (2) The system combines information from protein structures with functional annotations from a number of relevant databases and reports protein features (e.g., functional domains, known somatic variation in different types of cancer, UniProt annotations from missense variants, ligand binding residues, catalytic sites) that overlap the variant's 'direct matches' or their 'neighbors' in close physical proximity. These are defined by being within 5 angstroms spatial distance or adjacent in the sequence if no PDB covers that area.
- (3) When variants affect the interfaces of protein complexes (when the variant is at a distance of less than 8 angstroms from a residue in the partner protein), Structure-PPI also reports the partner proteins, and the residues in those proteins that are in close proximity to the variant.

Details: Manuscript > (BRAF)P15056-V600E

General Structures Structure-PPI Pathogenicity Databases Literature iHOP String

KinMut Random Forest
disease if score >0, neutral if score <0

| | | | |
|----------------------|-------|---------------------------|---------|
| Score | 0.669 | Prediction | disease |
| Group log-odds ratio | 0.629 | Kyte-Doolittle Hydroph... | 1 |
| GO log-odds ratio | 510 | Volume | 101 |
| C-beta branching | 0 | Formal charge | 0 |
| FireDB residue | No | PhosphoELM residue | No |
| Uniprot residue | No | Active site | No |
| Binding | No | Carbohydrate | No |
| Disulfide | No | Metal | No |
| Modified residue | No | NP Binding | No |
| Repeat | No | Signal | No |
| Site | No | Trans-membrane | No |
| Zinc finger | No | | |

Affected protein domains

| Domain | Log odds ratio |
|----------------|----------------|
| Protein_kinase | 0.950917 |

- (1) wKinMut-2 implements an ad-hoc method specific to the protein kinase superfamily. We termed this new methodology KinMutRF. KinMutRF classifies variants as neutral or disease-associated. A score ranging from 0 to 1 provides a measure of the reliability of the prediction.
- (2) The method relies on a random forest classifier consisting of 26 decision trees that evaluate a number of sequence-derived features that characterize variants affecting human protein kinases at different levels: a) at the gene level, including membership to a Kinbase group and Gene Ontology categories; b) at the domain level, using PFAM domains; and c) at the residue level, involved amino acids types, changes in biochemical properties, functional annotations from UniProt, Phospho.ELM and FireDB. These features are provided to guide the interpretation of the pathogenicity predictions and to help draw hypotheses on the plausible biological mechanisms by which the pathogenicity arose.





| Mutation | Description | Database |
|------------------|---|--------------------------|
| V600E | somatic mutation in cancer | cosmic |
| V600E | passenger | greenman |
| V600E | driver | greenman |
| V600D | Polymorphism | uniprot |
| V600E | Disease: Colorectal cancer (CRC) [MIM:114500] | uniprot |
| BRAF_HUMAN.V600E | Freq. 192% Activating: Colorectal carcinoma, melanoma and many others | kindriver |

- (1) wKinMut-2 incorporates relevant information from the UniProt Variant Pages, KinMutBase, Kin-Driver, COSMIC and ClinVar. The information is intended to facilitate a digested contextual framework for the interpretation of consequences of the variants. Of particular interest, any experimental evidence relating variants and disease.

Disclaimer: Users of previous versions of the tool would notice that SAAPdb is not included in the current implementation, as its authors have discontinued it.

Details: Manuscript > (BRAF)P15056 - V600E

General Structures Structure-PPI Pathogenicity Databases Literature iHOP String

| Mutation | PMID | Content | Comments |
|----------|--------------------------|---|----------|
| V600E | 15009715 | The predominant BRAF mutation T1799A (V600E) was detected in 18 | |
| V600E | 15277467 | The T1799A (V600E) mutation was detected by sequencing in melanomas from 5 of 22 patients as well as in the positive control, a cutaneous melanoma cell | |
| V600E | 15294323 | Real-time allele-specific amplification for sensitive detection of the BRAF mutation | |
| V600E | 15294323 | We found the V600E mutation in four samples | |
| V600E | 15342696 | Detection of the V600E mutation in a colorectal MSI-H tumour argues against the presence of a germline mutation in either the MLH1 or MSH2 | |
| V600E | 15342696 | Therefore, screening of these mismatch repair (MMR) genes can be avoided in cases positive for V600E if no other significant evidence, such as fulfilment of the strict Amsterdam criteria, suggests MMR associated | |
| V600E | 15342696 | Recently, an oncogenic V600E hotspot mutation within BRAF, a kinase encoding gene from the RAS/RAF/MAPK pathway, has been found to be associated with sporadic MSI-H colon cancer, but its association with HNPCC remains to be further | |

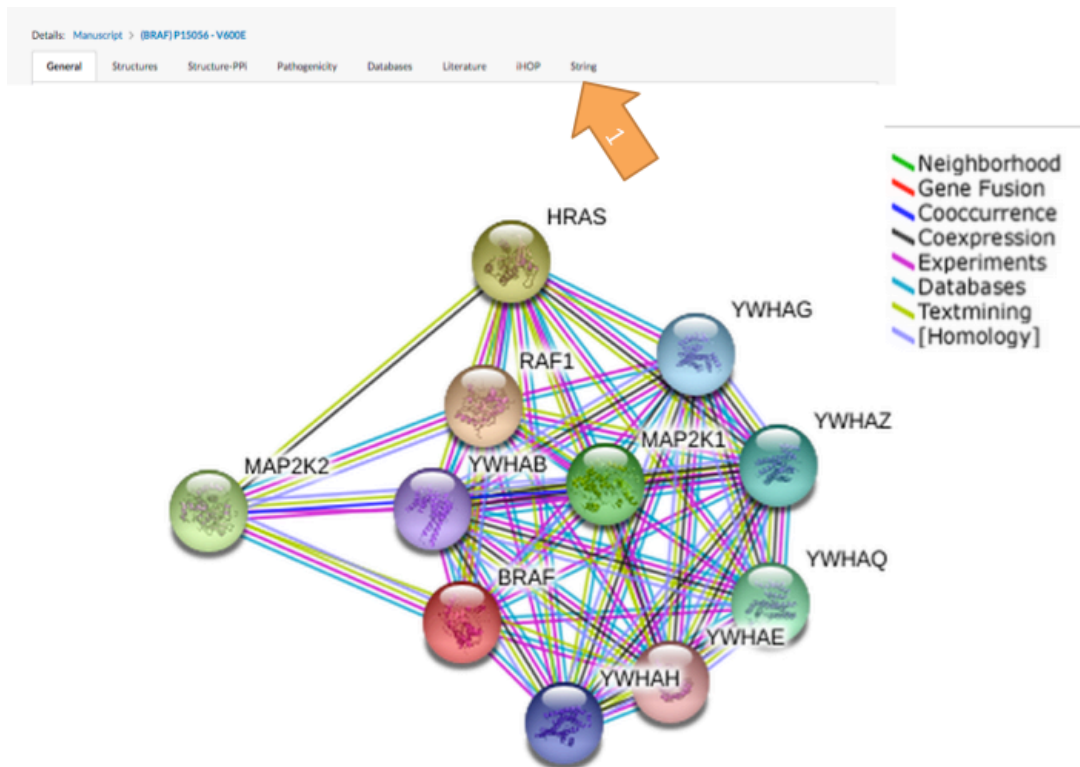
- (1) In an attempt to complement the information provided by the databases, wKinMut-2 provides information mined directly from the literature (Pubmed abstract and full-texts) with SNP2L. It is often the case that relevant information about the experimental conditions, the patients in the cohort, etc, can be found in the literature although it is missing in the databases due to the particular constraints of its design. The full text articles should provide a deeper understanding of these individual peculiarities.
- (2) The system provides links to the original publications (PMIDs) and displays the specific sentences where the variants were mentioned.



Synonyms: B-RAF, B-raf, BRAF, V-RAF murine sarcoma viral oncogene homolog B1, p94, v-raf murine sarcoma viral oncogene homolog B1

- [PMID: 16953233] Mutated LN metastases displayed KRAS associated or not with **BRAF** mutations.
- [PMID: 18782444] These results support that **BRAF** mutations harbour a mild oncogenic effect in comparison to KRAS and suggest that **BRAF** mutant colorectal cells need to accumulate extra epigenetic alterations in order to acquire full transformation and evolve to MSI CRC.
- [PMID: 17119447] NRAS mutations were associated with a significantly higher Clark level of invasion ($P=0.022$) than **BRAF** mutations.
- [PMID: 16567964] In addition, although **BRAF** and NRAS mutations are mutually exclusive in melanomas, other genetic events may complement **BRAF** mutation to produce biological activity similar to NRAS mutation.
- [PMID: 20140953] 3. Reduced gene dosage at MTAP showed a borderline association with **BRAF** mutation ($P = 0.04$) and reduced gene dosage at the interferon gene cluster was borderline associated with wild type NRAS ($P = 0.05$).
- [PMID: 16964246] Specifically, we found that oncogenic forms of HRAS (HRAS(G12V)) but not its downstream target **BRAF** (BRAF(V600E)), engaged a rapid cell-cycle arrest that was associated with massive vacuolization and expansion of the ER.
- [PMID: 15791648] Here, we examined the effects of cancer-related **B-raf** mutations surrounding Thr439 on the activation of the

(1) Similarly, wKinMut-2 sources literature co-mentions from iHOP. Literature co-mentions constitute a good proxy to interactions. In addition to the links to the original articles (PMIDs), the specific sentences in the literature are displayed. They are intended to provide contextual information that can facilitate the interpretation of the consequences of the pathogenic variation.



- (1) STRING is a resource that stores known and predicted protein interactions from different sources including genomic context, high-throughput experiments, coexpression and text-mining of the literature. STRING interactions include direct (physical) and indirect (functional) association.