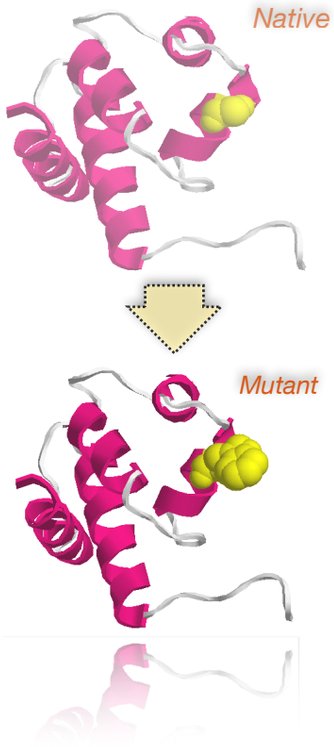
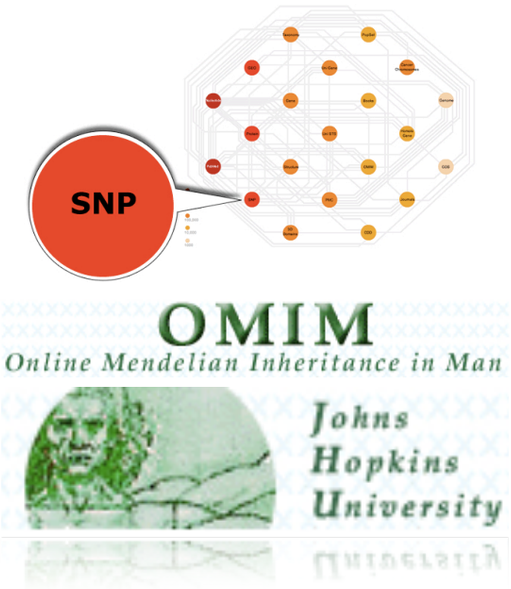
Computational methods for genome interpretation

MCCMB 2021

Monday August 2nd, 2021





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http://biofold.org/

Biomolecules
Folding and
Disease

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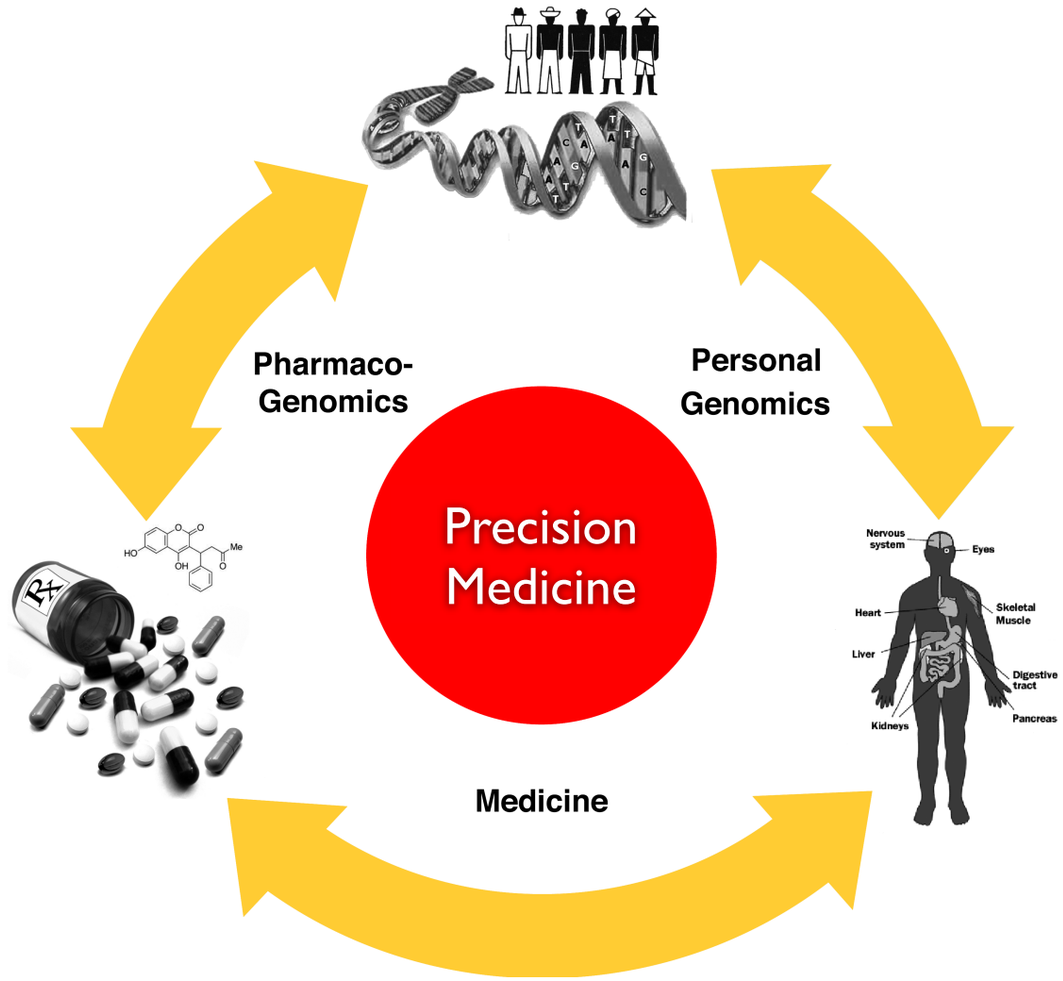
Presentation outline

- Introduction: Precision Medicine and Variant interpretation.
- Protein variants: sequence and structural features.
- Meta prediction: selection of highly-accurate predictions.
- Impact of noncoding variants: conservation in noncoding regions.
- Prediction assessment: The CAGI experiments.

Precision medicine

In the last decade, the cost of a whole genome sequencing experiment dropped below $1000. The increasing amount of sequencing data is raising important bioinformatics challenges.

1. Robust sequencing data processing methods
2. Interpretation of the functional effect and the impact of genomic variations
3. Integrating the molecular mechanisms and data to capture complexity of the system
4. Make the data clinically relevant



Fernald GH, et al (2011). Bioinformatics. 27: 1741-1748.

Single Nucleotide Variants

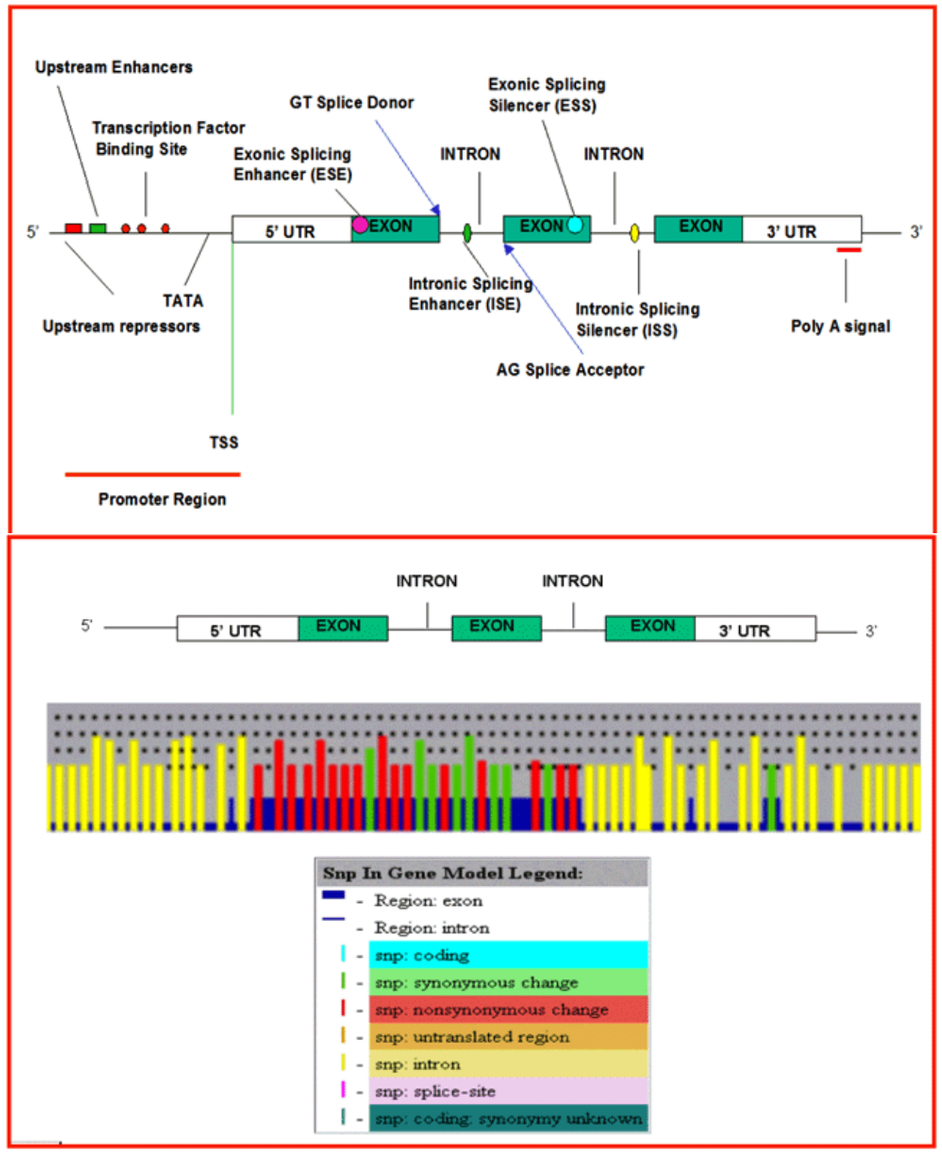
Single Nucleotide Variants (SNVs)
is a DNA sequence variation occurring when a single nucleotide A, T, C, or G in the genome differs between members of the species.
It is used to refer to Polymorphisms when the population frequency is ≥ 1%

SNVs occur at any position and can be classified on the base of their locations.

Coding SNVs can be subdivided into two groups:

Synonymous: when single base substitutions do not cause a change in the resultant amino acid

Non-synonymous or Single Amino Acid Variants (SAVs): when single base substitutions cause a change in the resultant amino acid.



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

1000 Genomes

The 1000 Genomes Project aims to create the largest public catalogue of human variations and genotype data. Last version released the genotype of ~2,500 individuals.

Table 1 | Variants discovered by project, type, population and novelty

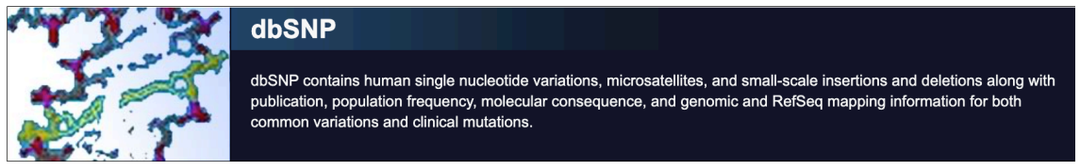
a Summary of project data including combined exon populations

	Low coverage				Trios				
Statistic	CEU	YRI	CHB+JPT	Total	CEU	YRI	Total	Exon (total)	Union across projects
Samples	60	59	60	179	3	3	6	697	742
Total raw bases (Gb)	1,402	874	596	2,872	560	615	1,175	845	4,892
Total mapped bases (Gb)	817	596	468	1,881	369	342	711	56	2,648
Mean mapped depth (×)	4.62	3.42	2.65	3.56	43.14	40.05	41.60	55.92	NA
Bases accessed (% of genome)	2.43 Gb (86%)	2.39 Gb (85%)	2.41 Gb (85%)	2.42 Gb (86.0%)	2.26 Gb (79%)	2.21 Gb (78%)	2.24 Gb (79%)	1.4 Mb	NA
No. of SNPs (% novel)	7,943,827 (33%)	10,938,130 (47%)	6,273,441 (28%)	14,894,361 (54%)	3,646,764 (11%)	4,502,439 (23%)	5,907,699 (24%)	12,758 (70%)	15,275,256 (55%)
Mean variant SNP sites per individual	2,918,623	3,335,795	2,810,573	3,019,909	2,741,276	3,261,036	3,001,156	763	NA
No. of indels (% novel)	728,075 (39%)	941,567 (52%)	666,639 (39%)	1,330,158 (57%)	411,611 (25%)	502,462 (37%)	682,148 (38%)	96 (74%)	1,480,877 (57%)
Mean variant indel sites per individual	354,767	383,200	347,400	361,669	322,078	382,869	352,474	3	NA
No. of deletions (% novel)	ND	ND	ND	15,893 (60%)	6,593 (41%)	8,129 (50%)	11,248 (51%)	ND	22,025 (61%)
No. of genotyped deletions (% novel)	ND	ND	ND	10,742 (57%)	ND	ND	6,317 (48%)	ND	13,826 (58%)
No. of duplications (% novel)	259 (90%)	320 (90%)	280 (91%)	407 (89%)	187 (93%)	192 (91%)	256 (92%)	ND	501 (89%)
No. of mobile element insertions (% novel)	3,202 (79%)	3,105 (84%)	1,952 (76%)	4,775 (86%)	1,397 (68%)	1,846 (78%)	2,531 (78%)	ND	5,370 (87%)
No. of novel sequence insertions (% novel)	ND	ND	ND	ND	111 (96%)	66 (86%)	174 (93%)	ND	174 (93%)

1000 Genomes Project Consortium (2010). Nature. 467: 1061-1073.

Variant databases

dbSNP @ NCBI

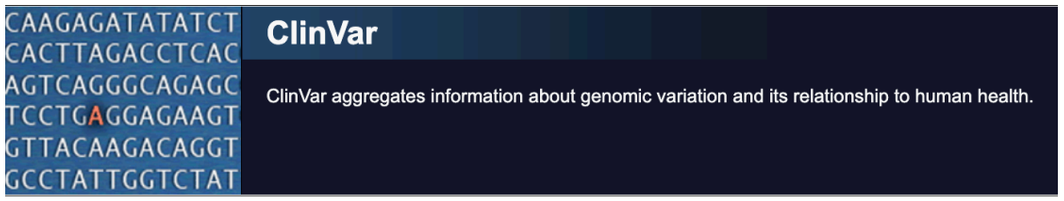


http://www.ncbi.nlm.nih.gov/snp

Single Nucleotide Variants

Homo sapiens	917,705,245

Clinvar @ NCBI



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

Single Nucleotide Variants

Homo sapiens	872,786
Pathogenic	*58,167*
Benign	*119,050*

humsavar @ UniProt

https://www.uniprot.org/docs/humsavar

Single Amino acid Variants

Homo sapiens	79,745
Pathogenic	*31,398*
Benign	*39,584*

Jul 2021

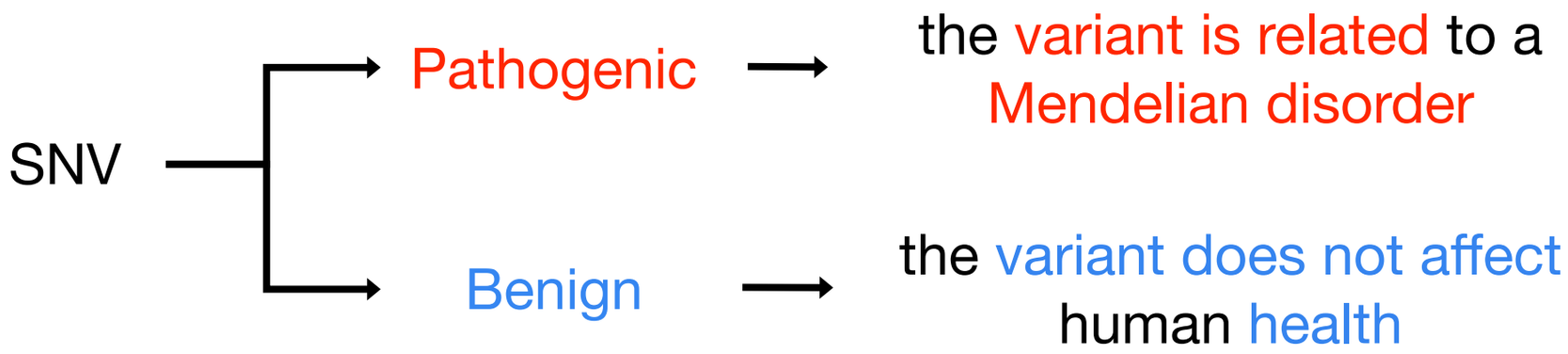
Effects of variants

Impact of coding variants

- Physico-chemical properties of the substituted residue
- Evolutionary important residues in specific protein sites
- Sequence–function relationships
- Structure–function relationships

Impact of noncoding variants

- Transcription
- Pre-mRNA splicing
- MicroRNA binding
- Altering post-translational modification sites



Cline and Karchin (2011) Bioinformatics, 27; 441-448.

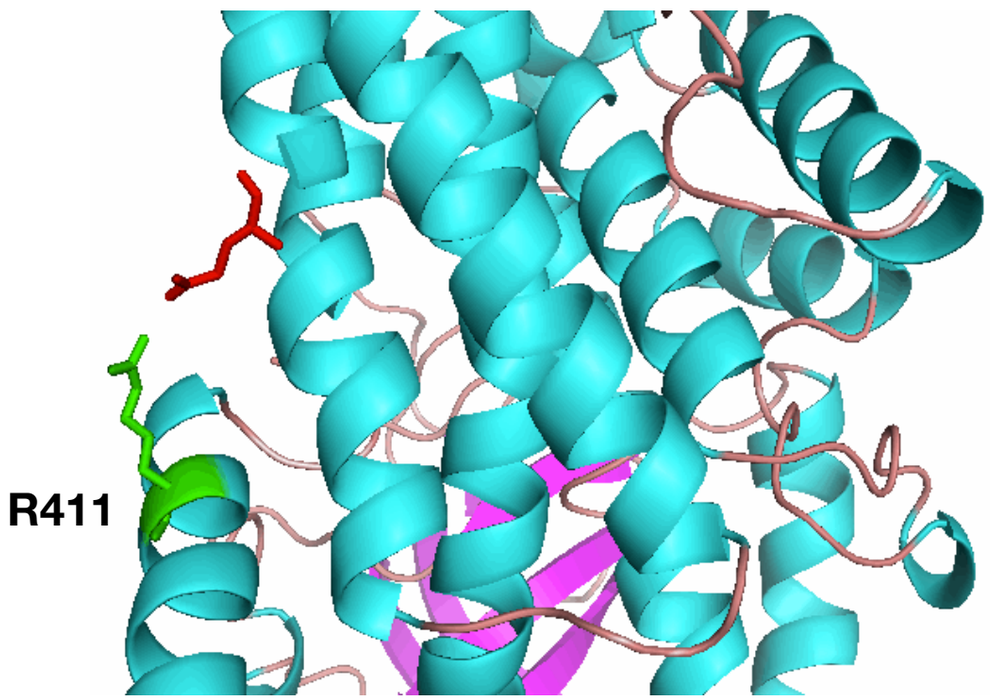
Protein variants

Sequence, Structure & Function

Genomic variants in sequence motifs can affect protein function.
Mutation S362A of P53 affect the interaction with hydrolase USP7 and the deubiquitination of the protein.

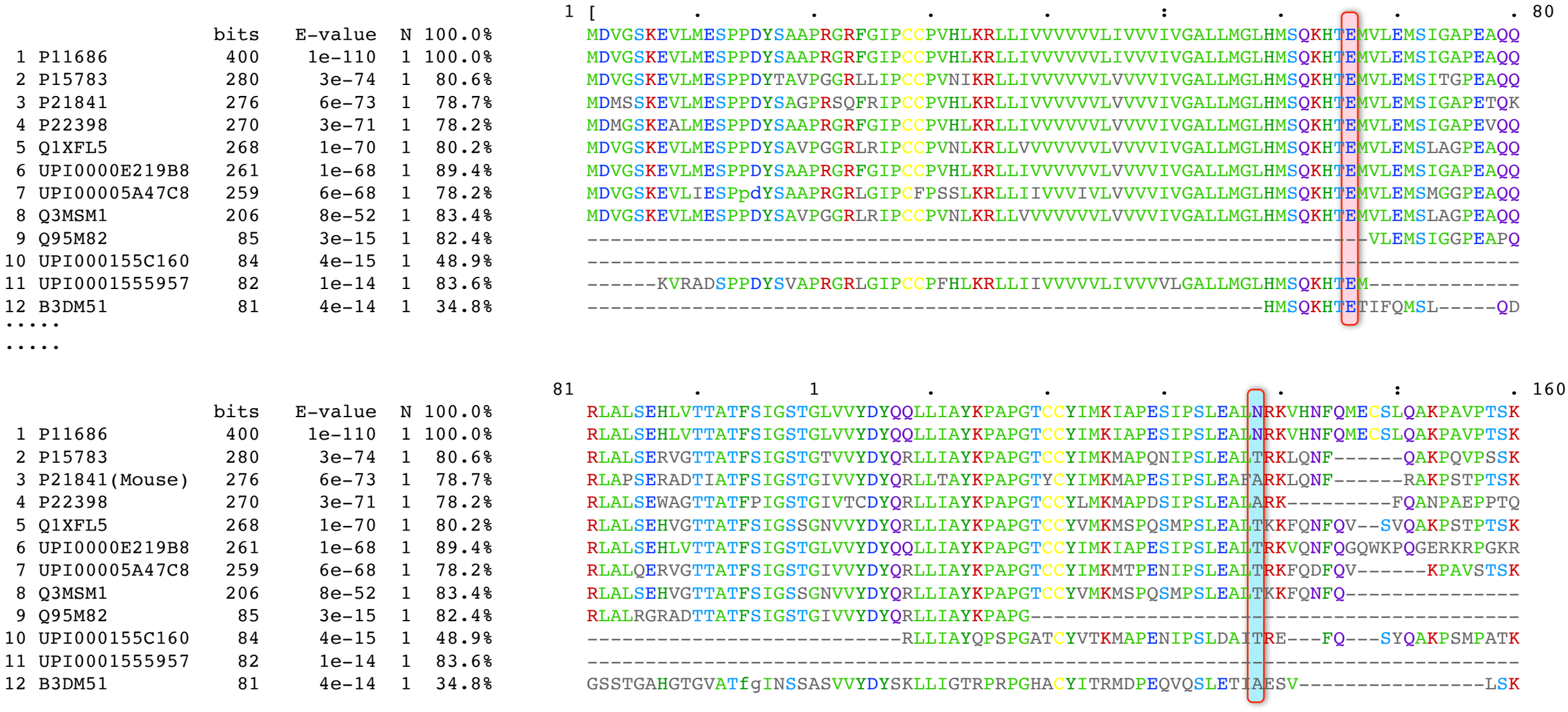
A nonsynonymous variant can affect the protein structure causing the loss of stability of the protein.

Mutation R411L results in the loss of a salt bridge, destabilizing the structure of the IVD dehydrogenase.



Conserved or not?

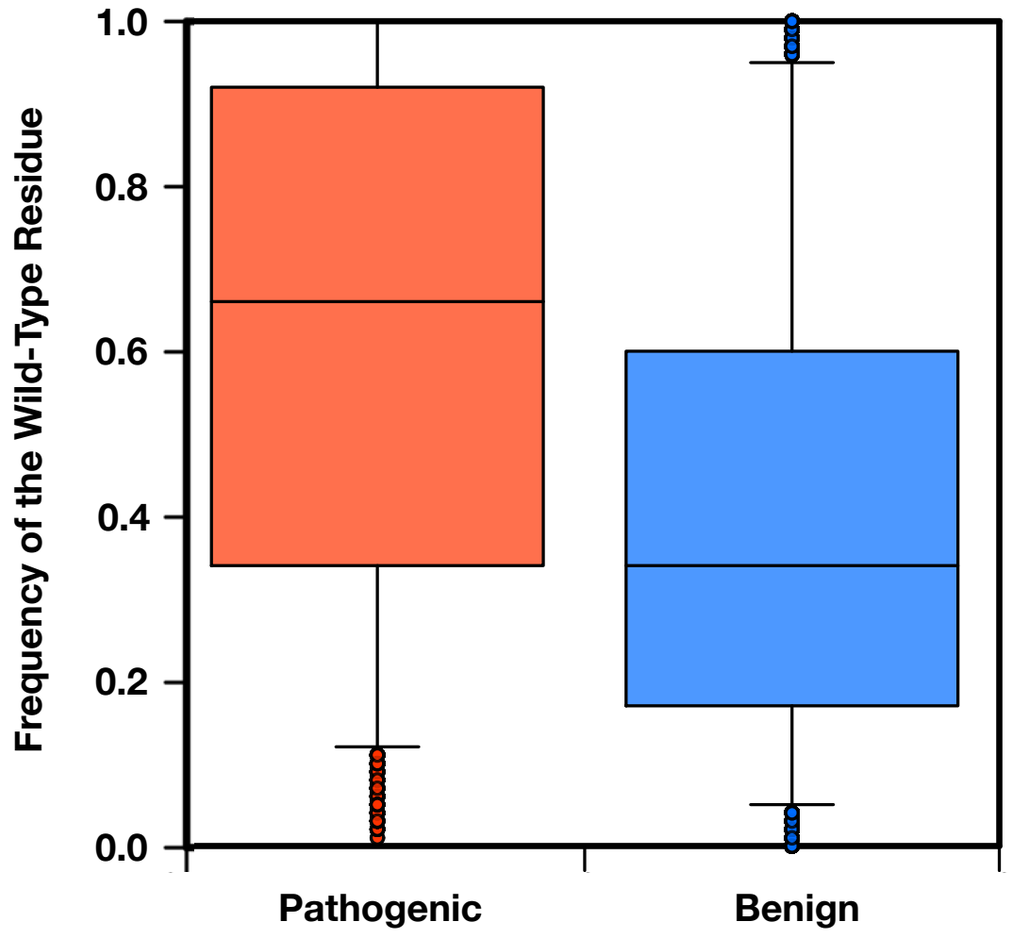
In positions 66 the Glutamic acid is highly conserved Asparagine in position 138 is mutated Threonine or Alanine



Sequence profile

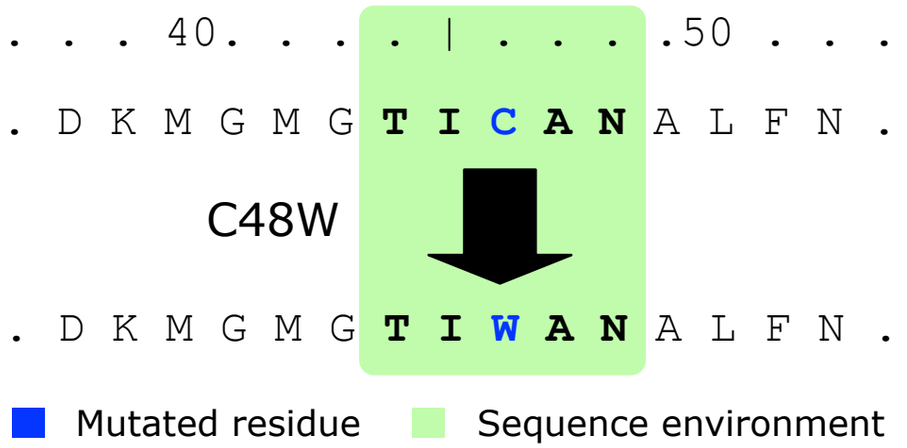
The protein sequence profile is calculated running BLAST on the UniRef90 dataset and selecting only the hits with e-value < 10^{-9}.

The distributions of the frequency of the wild-type residues for Pathogenic and Benign variants are significantly different.

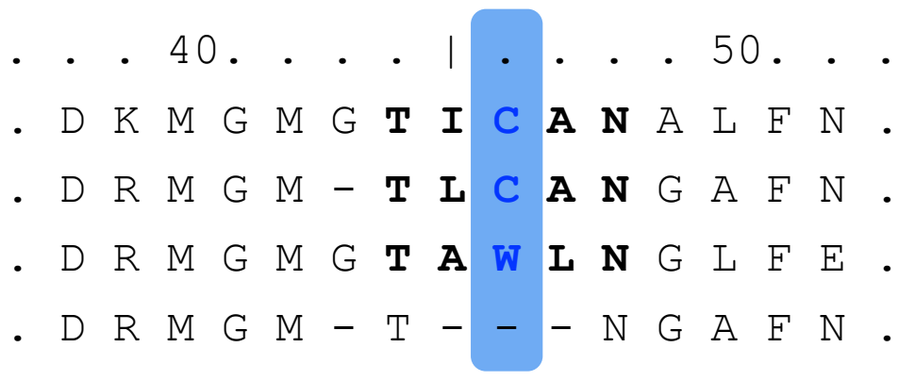


Capriotti et al (2012). Briefings in Bioinformatics. 13; 495-512.

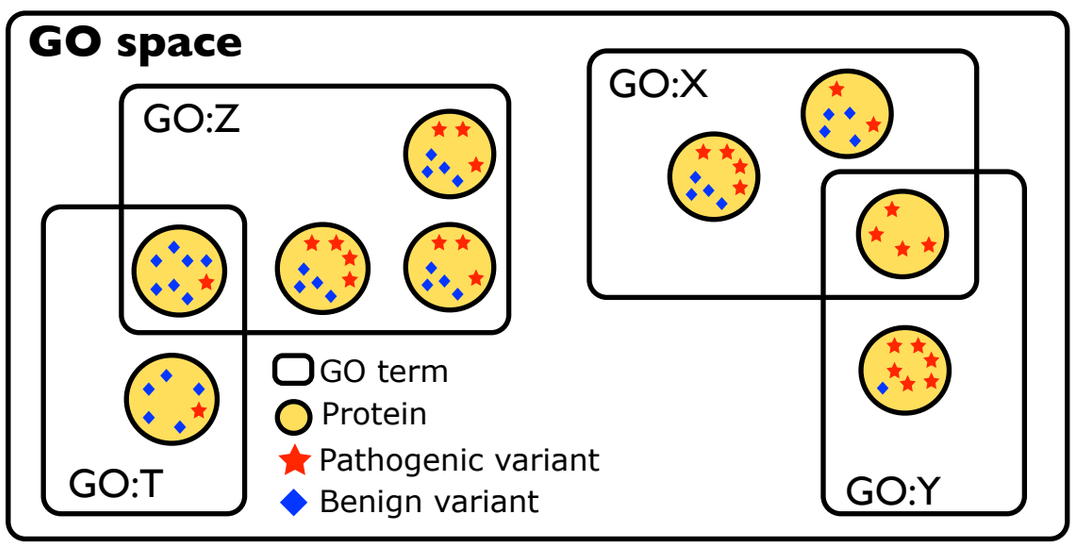
SNPs&GO input features



Sequence information is encoded in 2 vectors each one composed by 20 elements. The first vector encodes for the mutation and the second one for the sequence environment



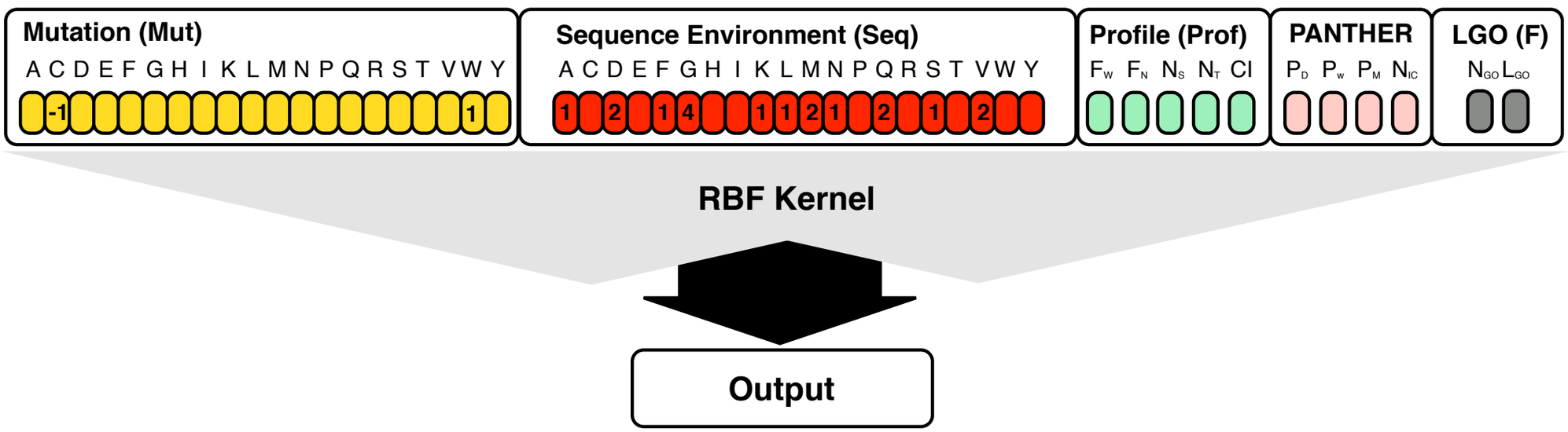
Protein sequence profile information derived from a multiple sequence alignment. It is encoded in a 5 elements vector corresponding to different features general and local features



The GO information are encoded in a 2 elements vector corresponding to the number unique of GO terms associated to the protein sequences and the sum of the logarithm of the total number of Pathogenic and Benign variants for each GO term.

SNPs&GO performance

SNPs&GO results in better performance with respect to previously developed methods.



Method	Q2	P[D]	Q[D]	P[N]	Q[N]	C	PM
PolyPhen	0.71	0.76	0.75	0.63	0.64	0.39	58
SIFT	0.76	0.75	0.76	0.77	0.75	0.52	93
PANTHER	0.74	0.77	0.73	0.71	0.76	0.48	76
SNPs&GO	0.82	0.83	0.78	0.80	0.85	0.63	100

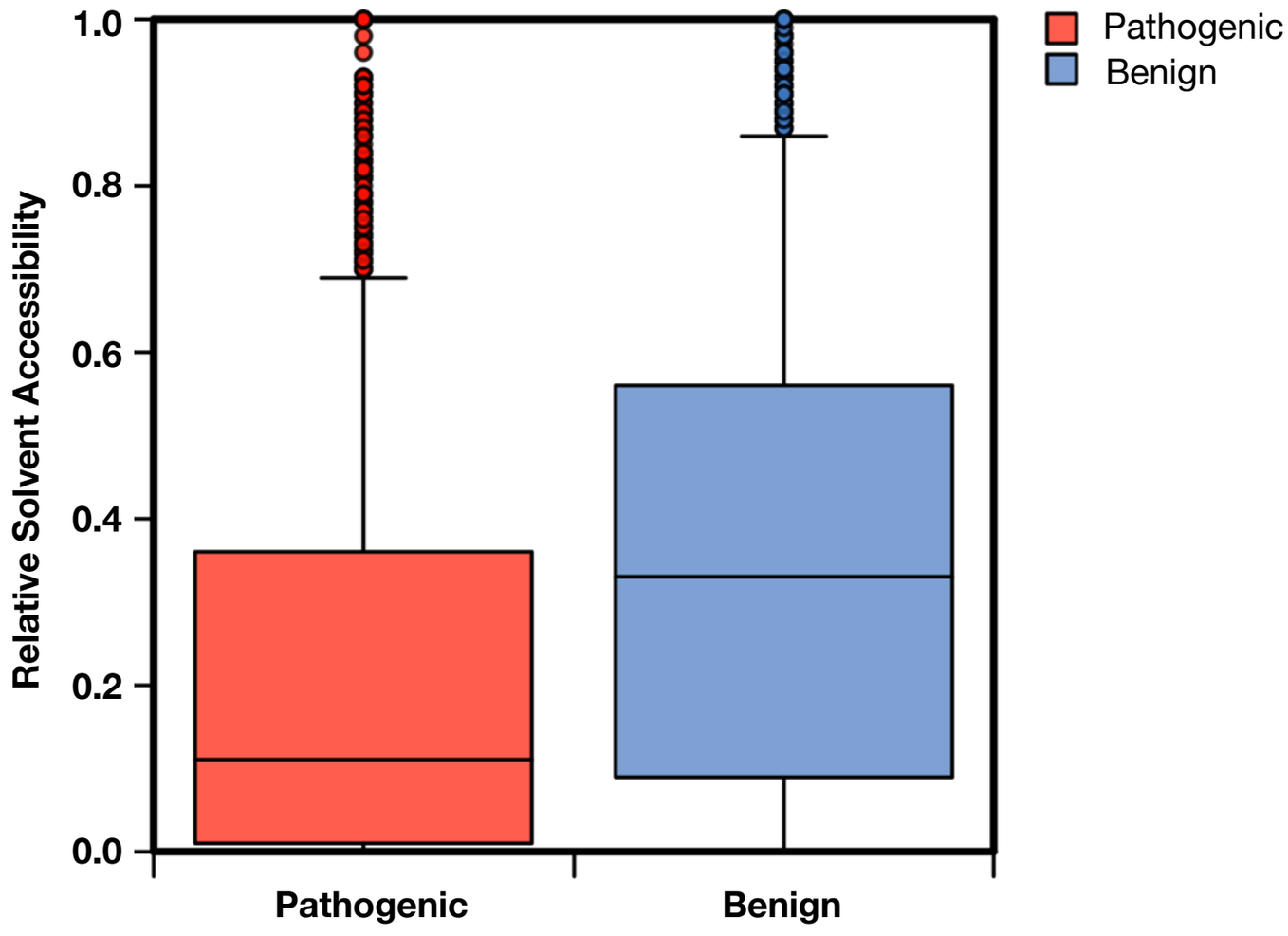
D = Pathogenic N = Benign

DB= 33672 nsSNVs

Calabrese et al. (2009) Human Mutation 30, 1237-1244.

Structure environment

There is a significant difference between the distributions of the Relative Solvent Accessibility for Pathogenic and Benign variants. The median values of their distributions are ~0.1 and 0.35 respectively.

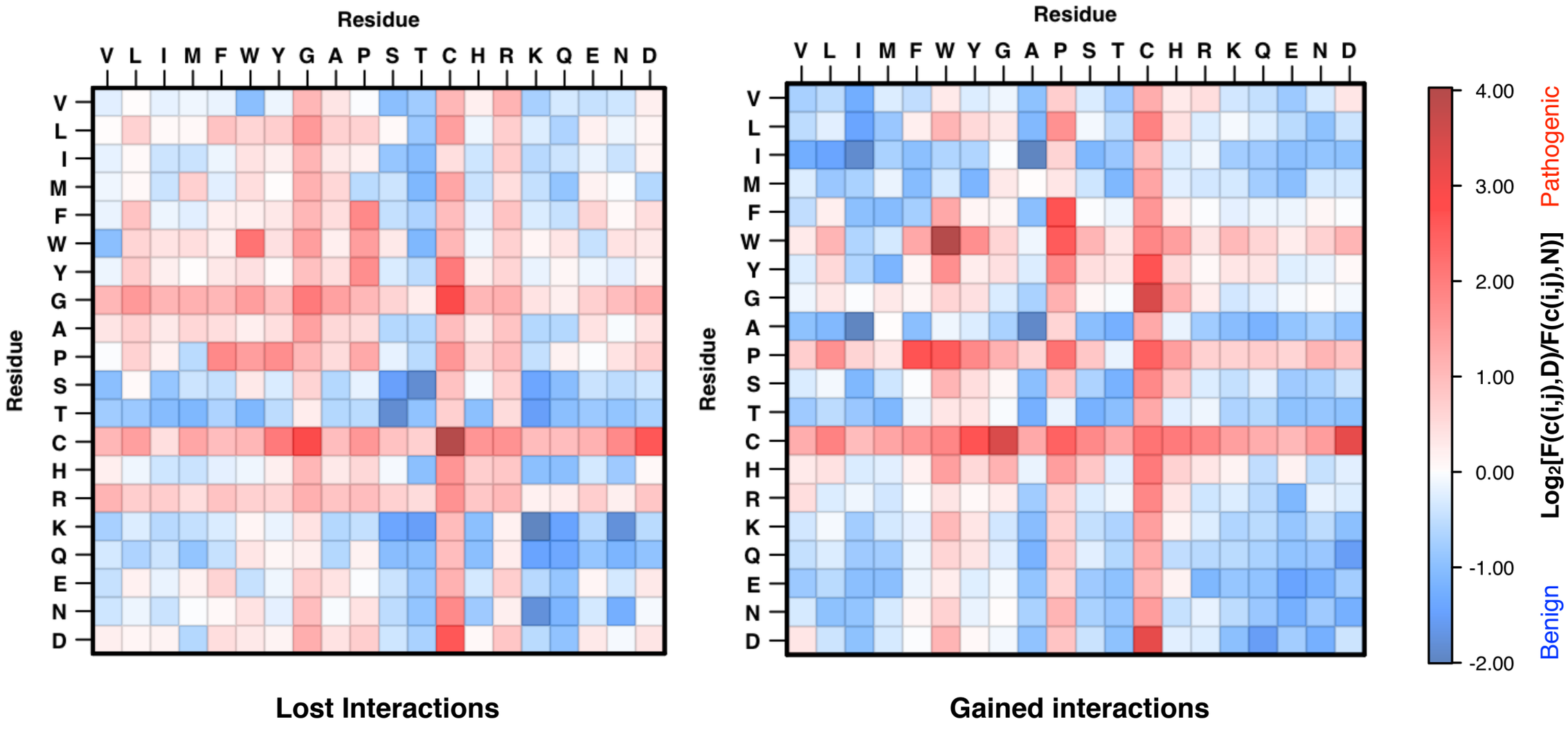


Capriotti and Altman. (2011) BMC Bioinformatics.12 (Suppl 4); S3.

Analysis of the 3D interactions

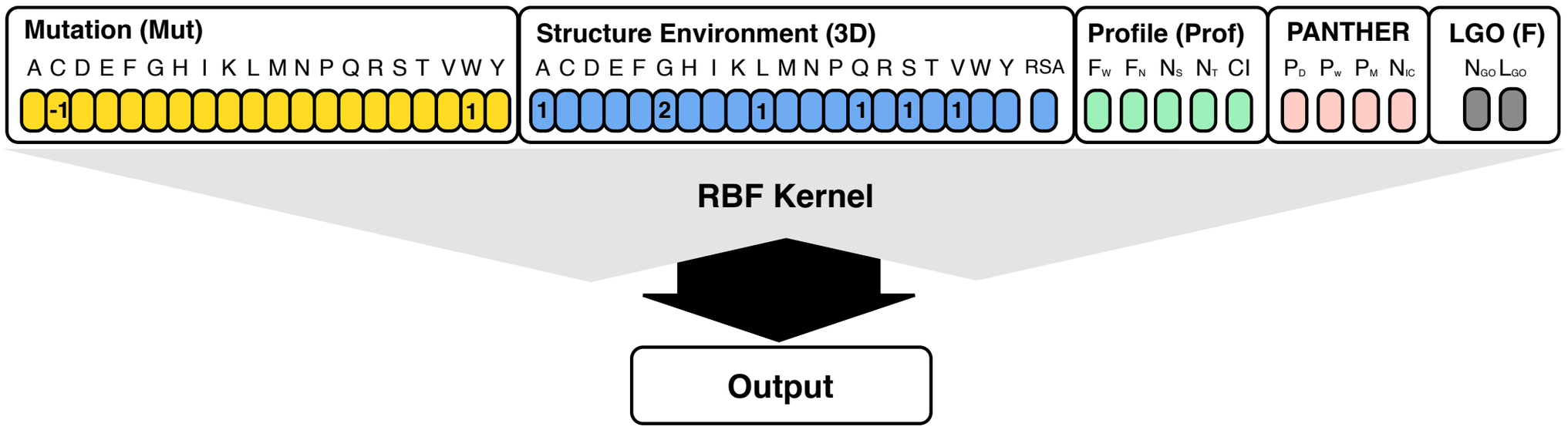
Using the whole set of SAVs with known structure, we calculate the log odd score of the ratio between the frequencies of the interaction between residue i and j for Pathogenic and Benign variants.

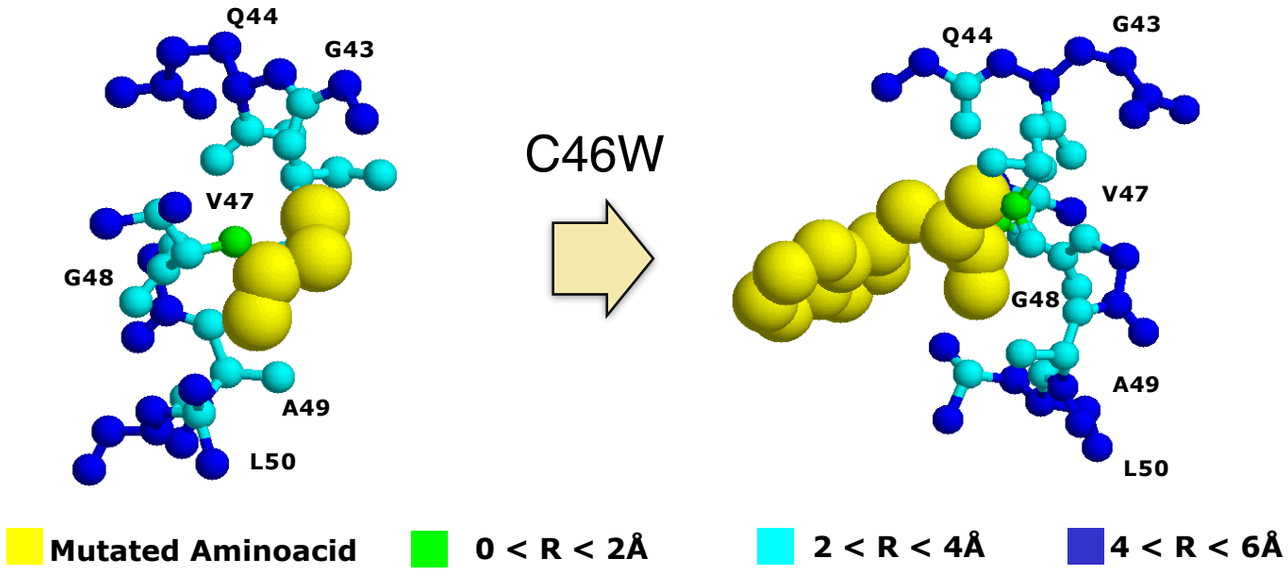
$$LC = \log_2 \left[\frac{n(i, j, Pathogenic) \,/\, N(Pathogenic)}{n\,(i, j, Benign) \,/\, N(Benign)} \right]$$



The structure-based method

The method takes as input a 52-element vector encoding for mutation; structure environment, sequence profile and functional score based on GO terms.

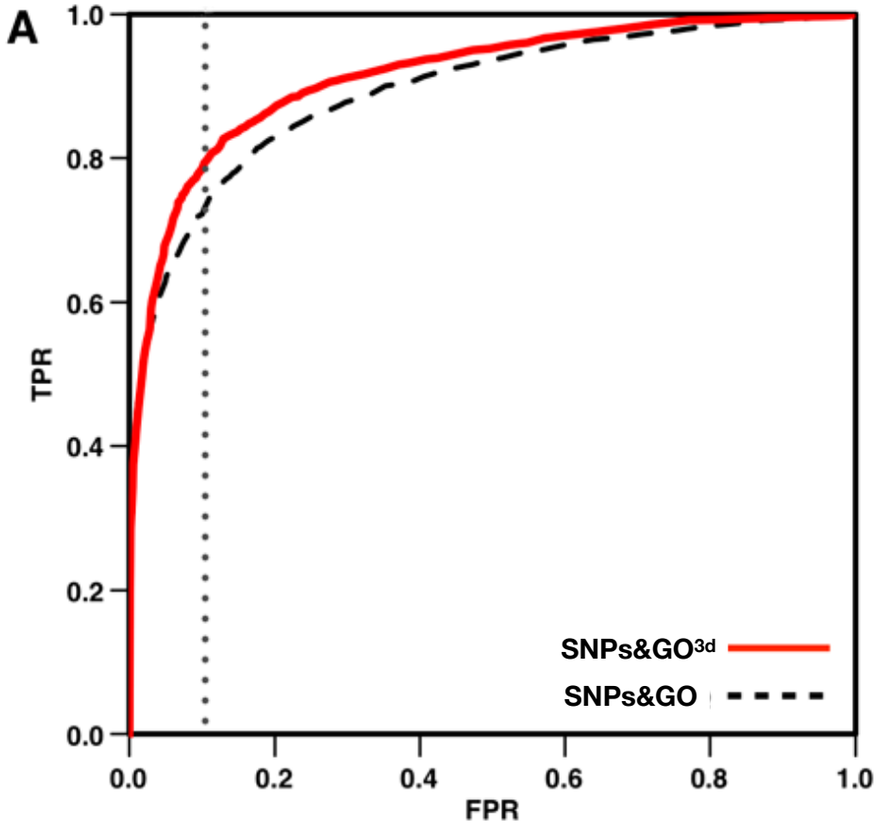


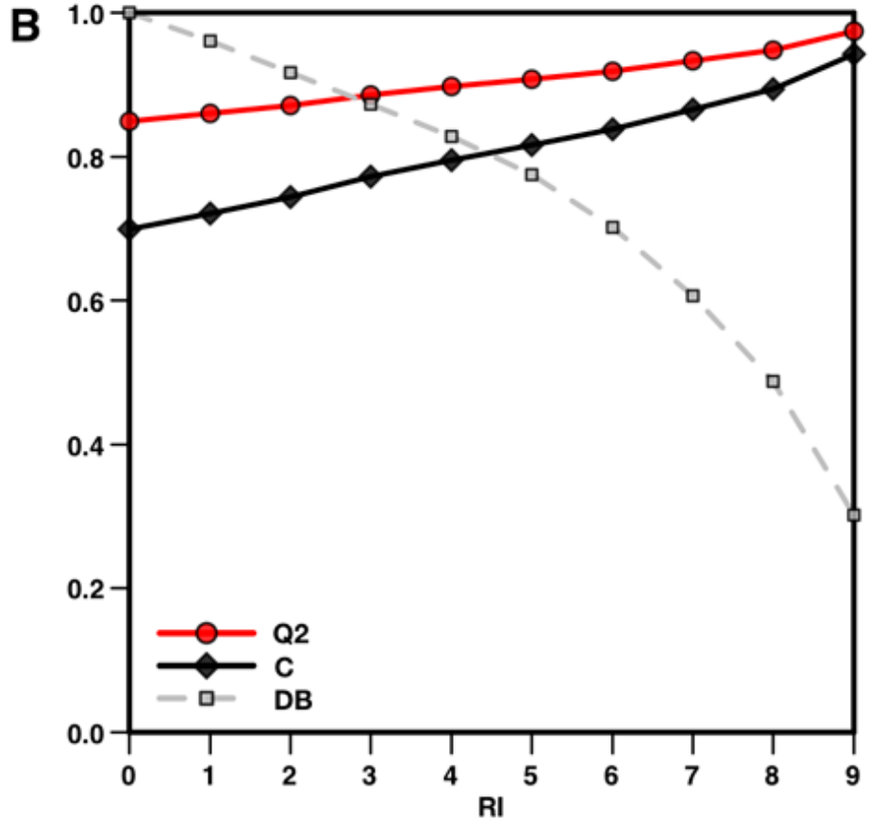


Sequence vs structure

The structure-based method results in better accuracy with respect to the sequence-based one. Structure based prediction are 3% more accurate and correlation coefficient increases of 0.06. If 10% of FP are accepted the TPR increases of 7%.

	Q2	P[D]	S[D]	P[N]	S[N]	C	AUC
SNPs&GO	0.82	0.81	0.83	0.82	0.81	0.64	0.89
SNPs&GO^{3d}	0.85	0.84	0.87	0.86	0.83	0.70	0.92



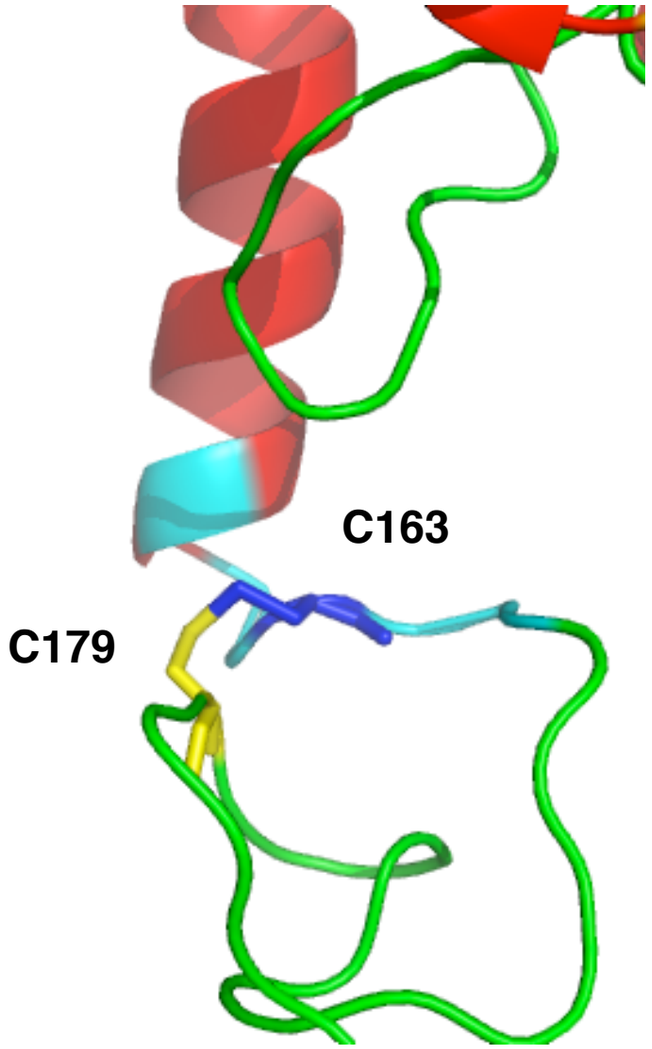


http://snps.biofold.org/snps-and-go

Prediction example

Damaging missing Cys-Cys interaction in the Glycosylasparaginase. The mutation p.Cys163Ser results in the loss of the disulfide bridge between Cys163 and Cys179. This amino acid variant is responsible for Aspartylglucosaminuria.

1APY: Chain A, Res: 2.0 Å



Meta prediction approach

Protein variant predictors

Many predictor of the effect of Single Amino acid Variants (SAVs) are available. They mainly use information from multiple sequence alignment to predict the effect of a given mutation. In this study we consider

- PhD-SNP: Support Vector Machine-based method using sequence and profile information (Capriotti et al. 2006).

- PANTHER: Hidden Markov Model-based method using a HMM library of protein families (Thomas and Kejariwal 2004).

- SNAP: Neural network based method to predict the functional effect of single poit mutations (Bromberg et al. 2008).

- SIFT: Probabilistic method based on the analysis of multiple sequence alignments (Ng and Henikoff 2003).

Predictors accuracy

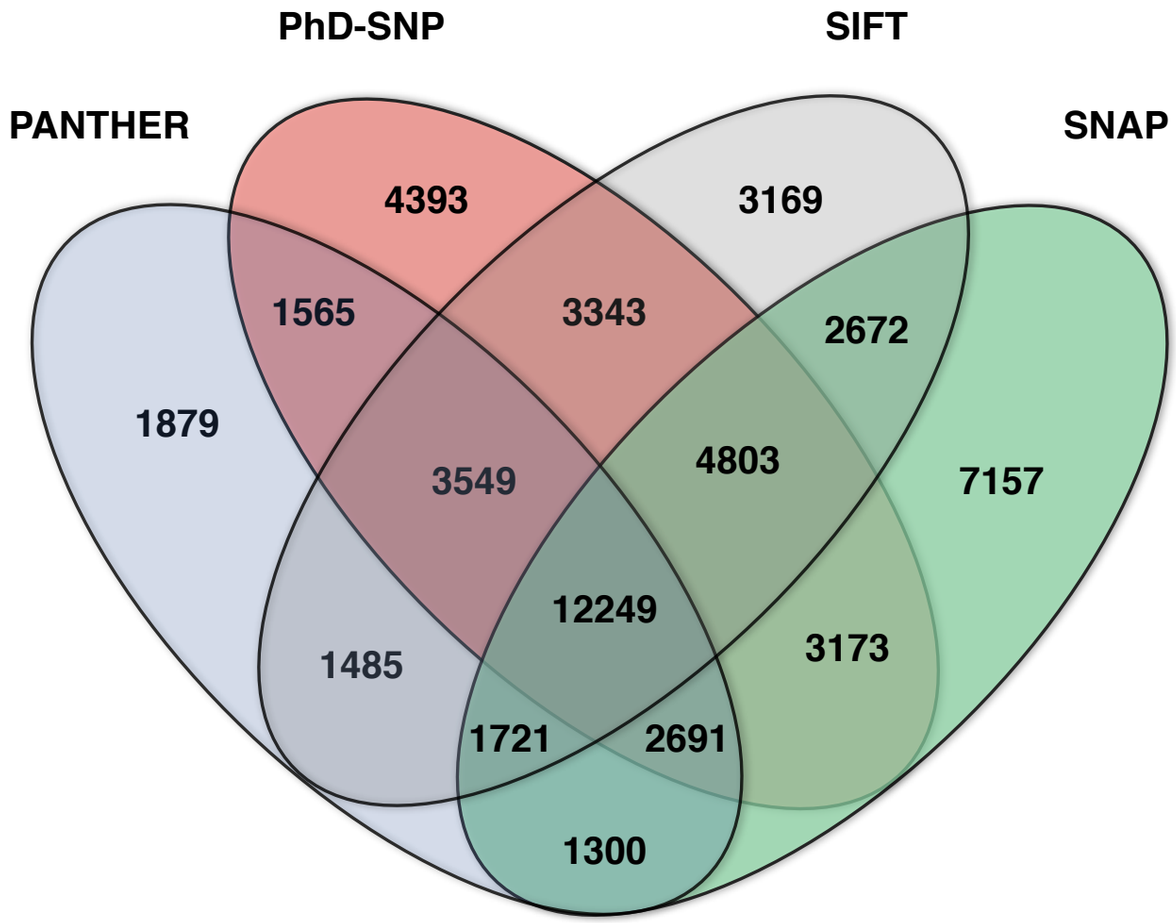
The accuracy of each predictor has been tested on a set of 35,986 mutations equally distributed between Pathogenic and Benign variants. PhD-SNP results in better accuracy but is the only one optimized using a cross-validation procedure.
SNAP shows lowest accuracy but it has been developed for a different task.

	Q2	P[D]	S[D]	P[N]	S[N]	C	PM
PhD-SNP	0.76	0.78	0.74	0.75	0.78	0.53	100
PANTHER	0.74	0.79	0.73	0.69	0.74	0.48	74
SNAP	0.64	0.59	0.90	0.79	0.38	0.33	100
SIFT	0.70	0.74	0.64	0.68	0.76	0.41	92

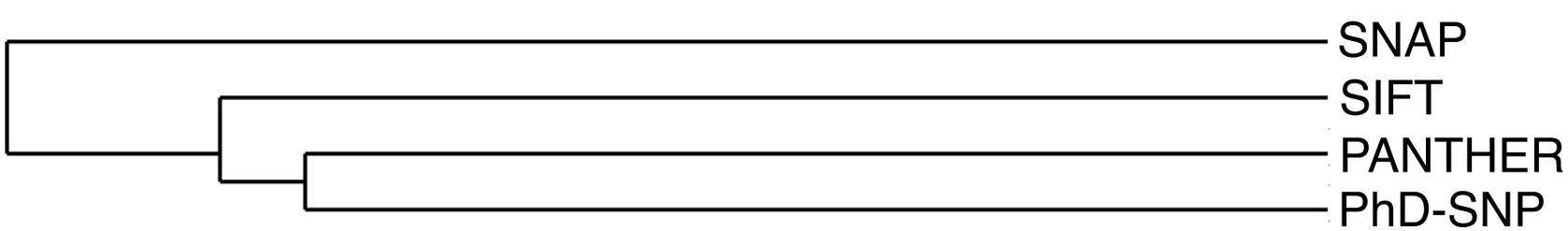
DB: Benign (N) 17883 and Pathogenic (D) 17883

Predictors tree

Using the prediction similarity we can build the predictors tree



UPGMA tree based on correlations



Prediction analysis

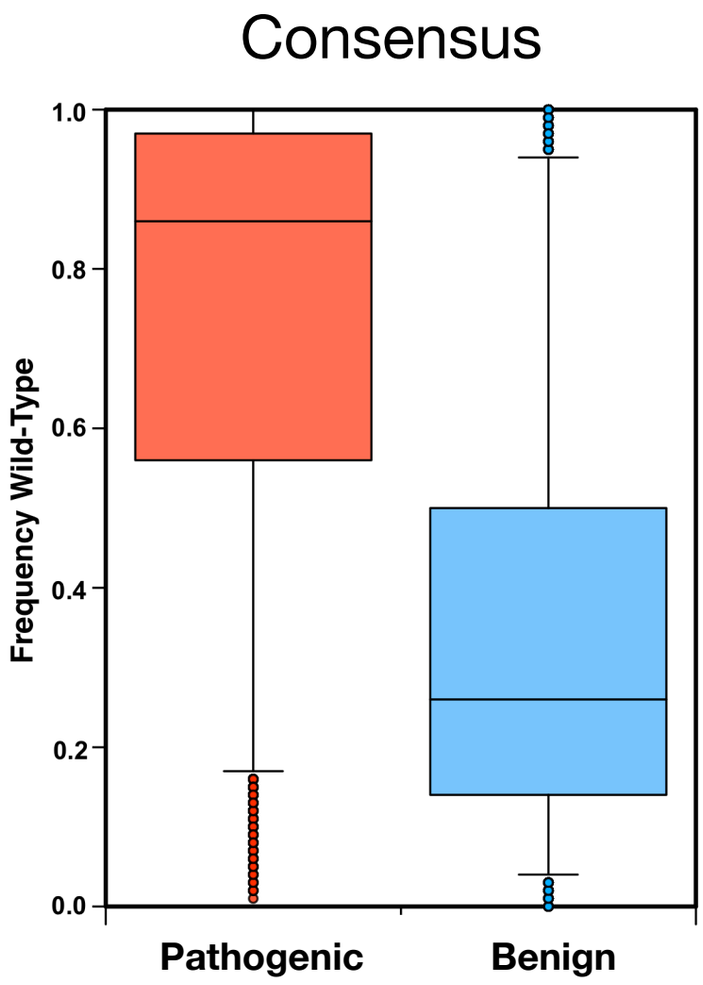
The accuracy of the predictions has been evaluated considering three different subset

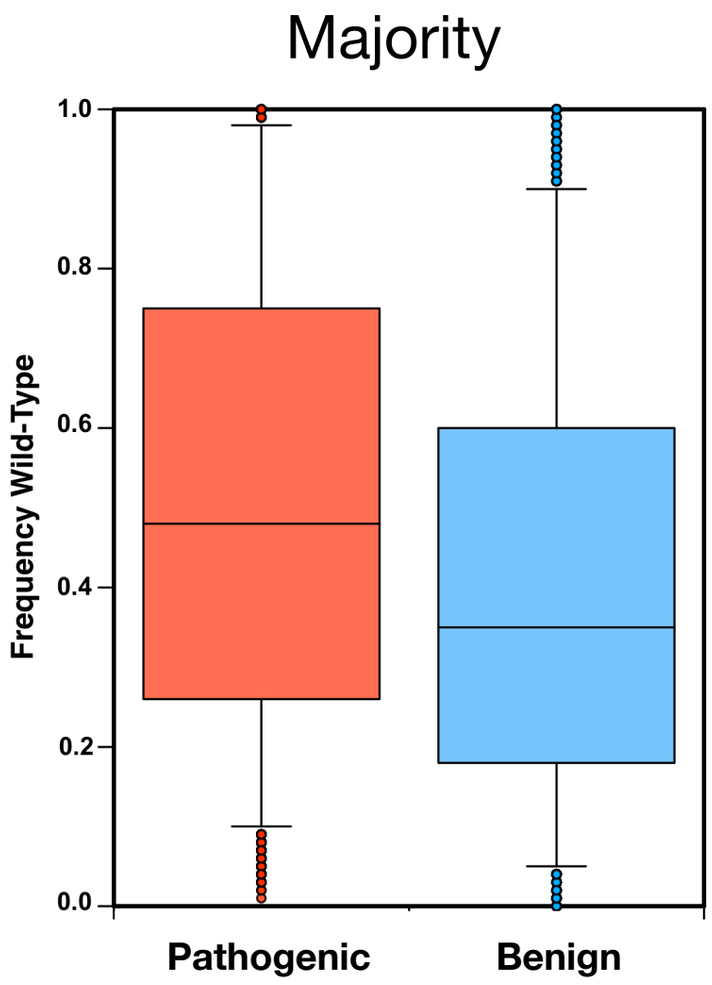
- Consensus: all the predictions returned by the methods are in agreement.
- Tie: equal number of methods predicting Pathogenic and Benign
- Majority: One of the two possible classes is predominant

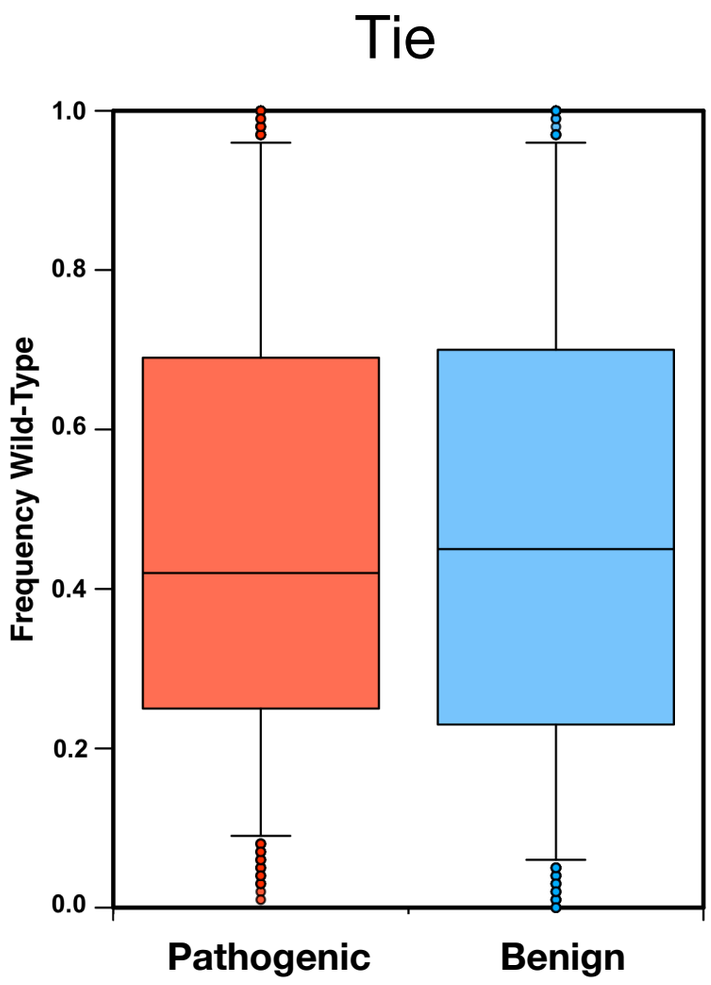
	Q2	P[D]	S[D]	P[N]	S[N]	C	AUC	%DB
PhD-SNP	0.76	0.78	0.74	0.75	0.78	0.53	0.84	100
Consensus	0.87	0.87	0.92	0.87	0.79	0.73	0.89	46
Majority	0.70	0.67	0.56	0.72	0.80	0.37	0.82	40
Tie	0.61	0.51	0.43	0.66	0.73	0.16	0.67	14

Subset conservation

The distributions of the wild-type frequencies for Pathogenic and Benign variants on the *Consensus* subset have very little overlap.



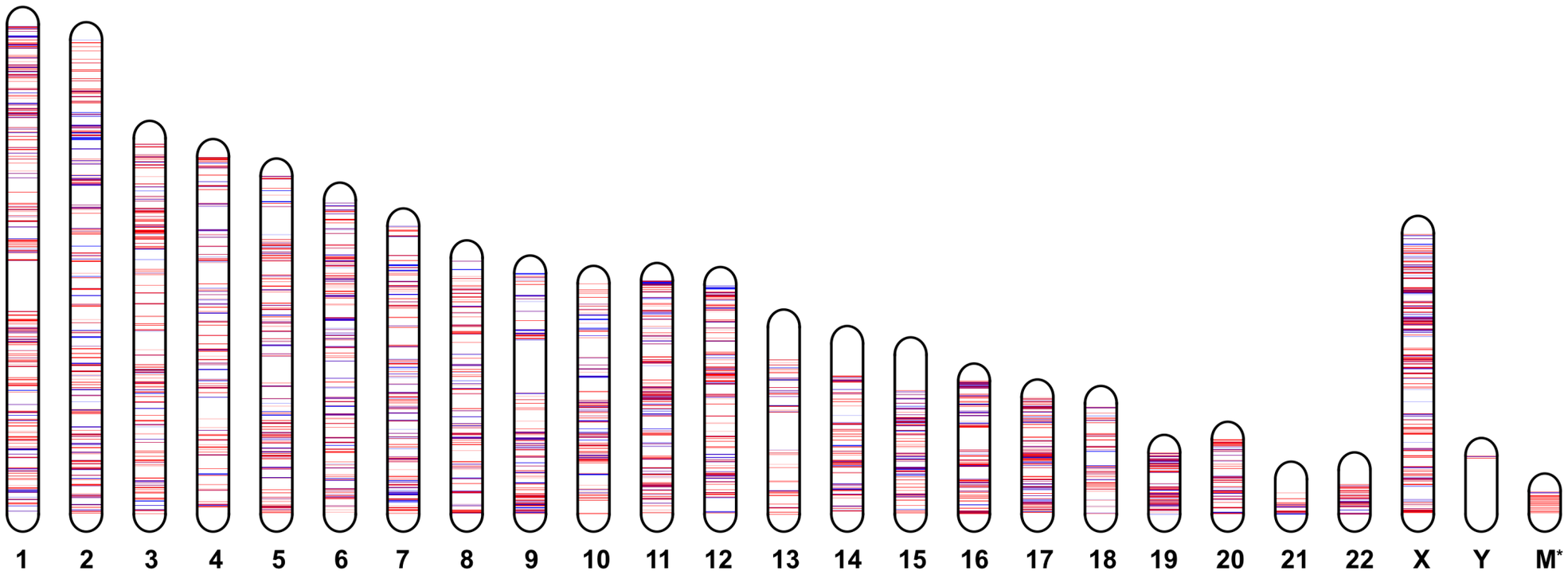




From coding to noncoding

Whole-genome predictions

Most of the genetic variants occur in noncoding region that represents >98% of the whole genome.

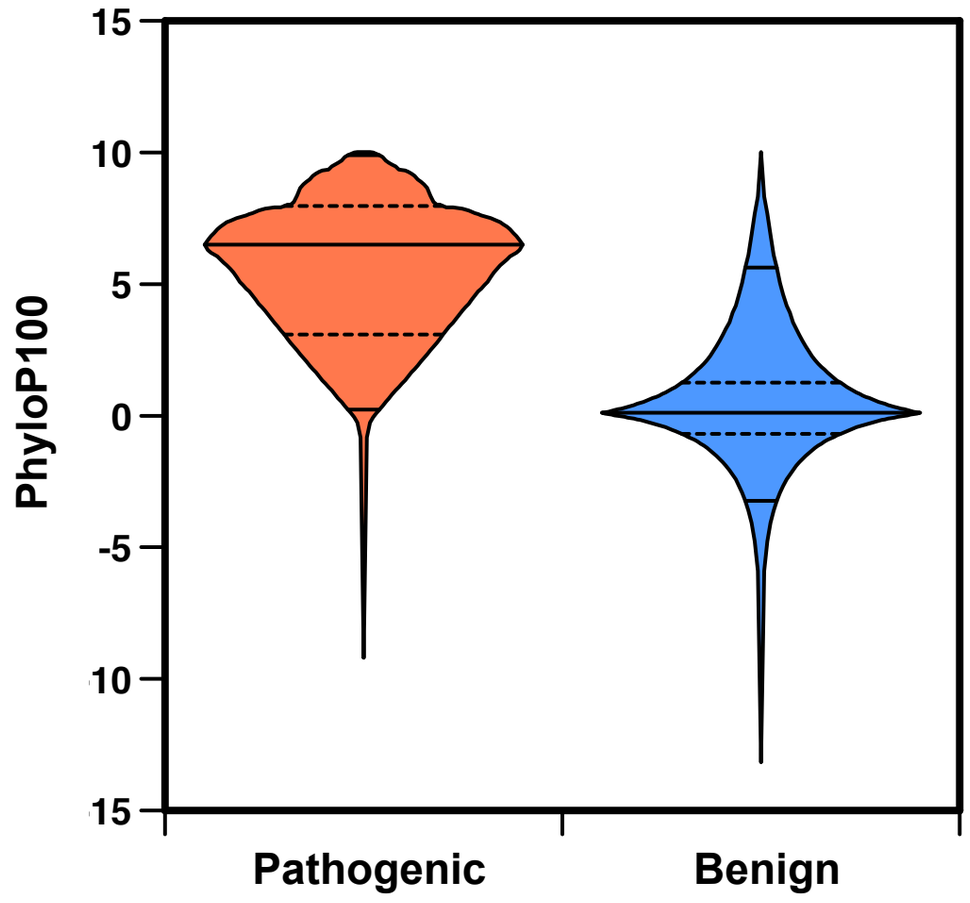


Predict the effect of SNVs in noncoding region is a challenging task because conservation is more difficult to estimate.

The sequence alignment is more complex task for noncoding regions.

PhyloP100 score

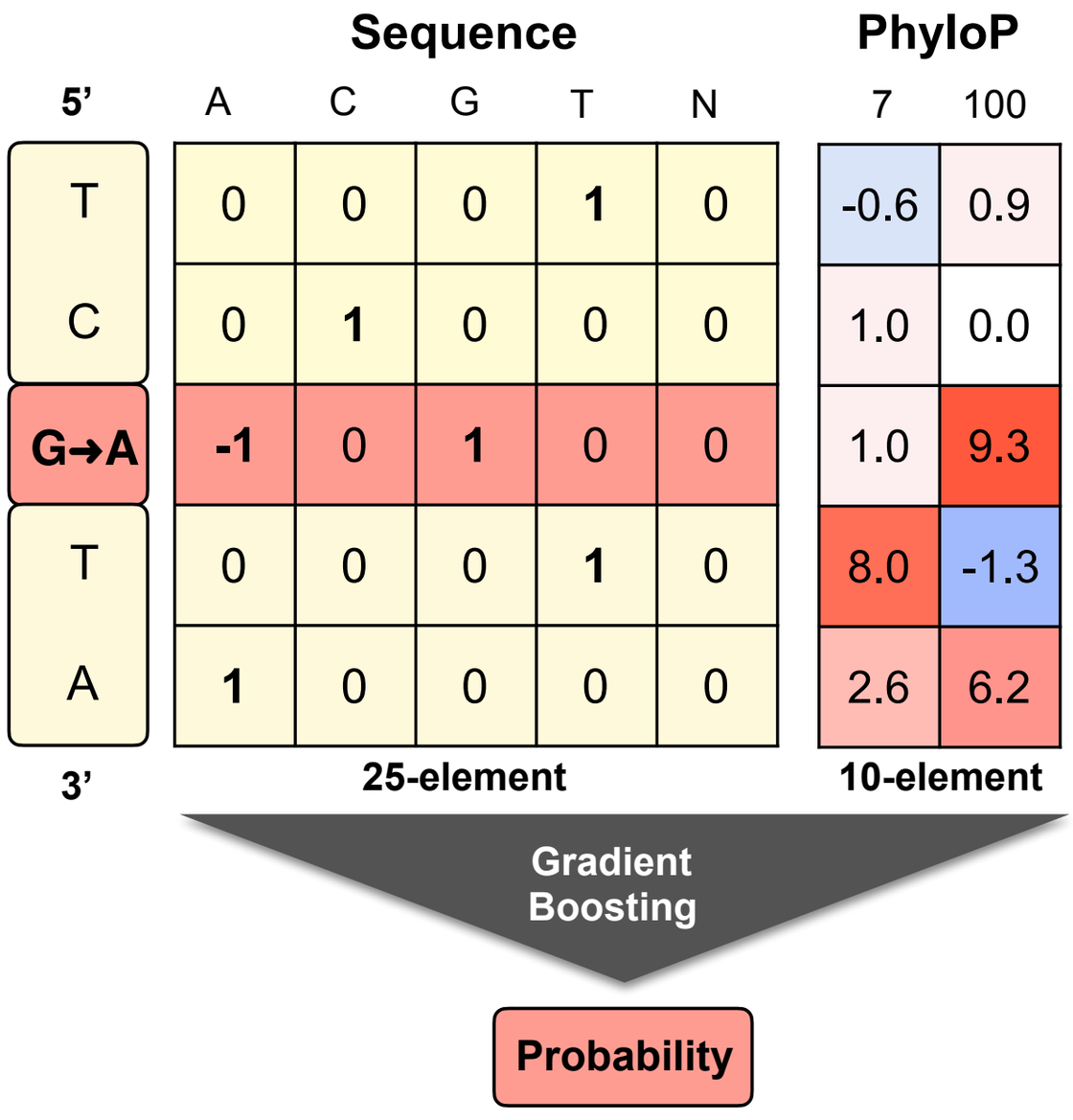
Conservation analysis based on the pre-calculated score available at the UCSC revealed a significant difference between the distribution of the PhyloP100 scores in Pathogenic and Benign SNVs.



PhyloP: Pollard et al,, Genome Research 2010

PhD-SNPg

PhD-SNPg is a simple method that takes in input 35 sequence-based features from a window of 5 nucleotides around the mutated position.

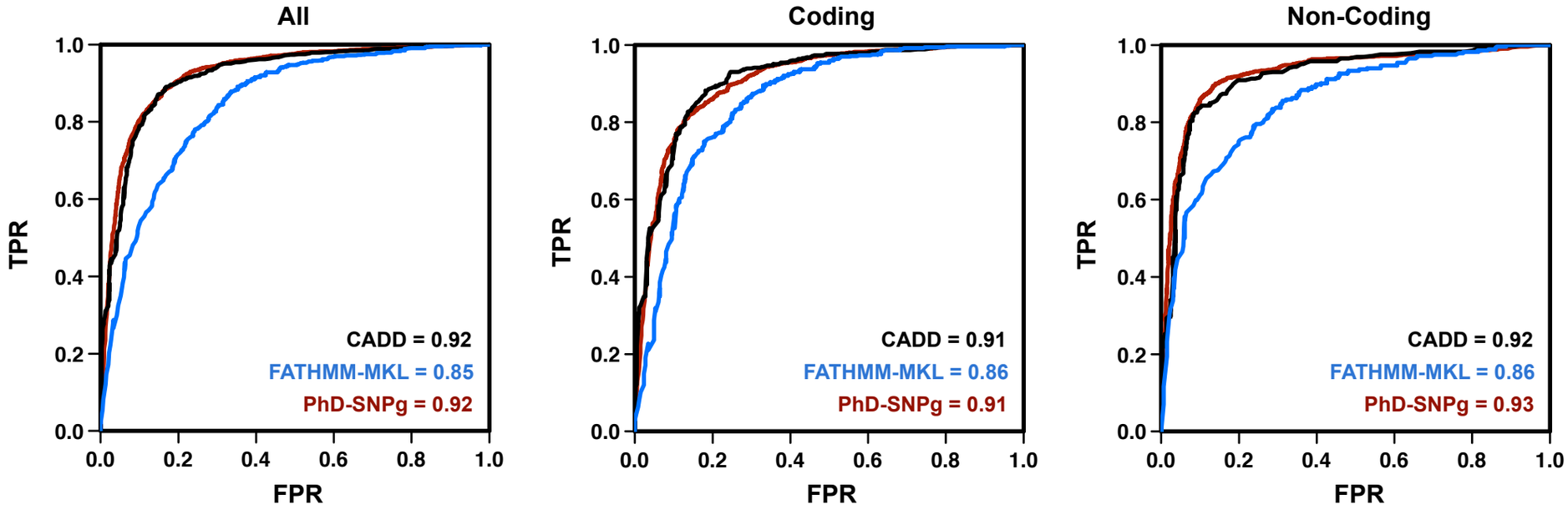


http://snps.biofold.org/phd-snpg/

Benchmarking

PhD-SNP^{g} has been tested in cross-validation on a set of 35,802 SNVs and on a blind set of 1,408 variants recently annotated.

	Q2	TNR	NPV	TPR	PPV	MCC	F1	AUC
PhD-SNP^{g}	0.861	0.774	0.884	0.925	0.847	0.715	0.884	0.924
Coding	0.849	0.671	0.845	0.938	0.850	0.651	0.892	0.908
Non-Coding	0.876	0.855	0.911	0.901	0.839	0.753	0.869	0.930



Capriotti and Fariselli. (2017) Nucleic Acids Res. PMID: 28482034.

Blind testing

CAGI experiments

The Critical Assessment of Genome Interpretation is a community experiment to objectively assess computational methods for predicting the phenotypic impacts of genomic variation.

https://genomeinterpretation.org/

The NAGLU challenge

NAGLU is a lysosomal glycohydrolyase which deficiency causes a rare disorder referred as Sanfilippo B disease

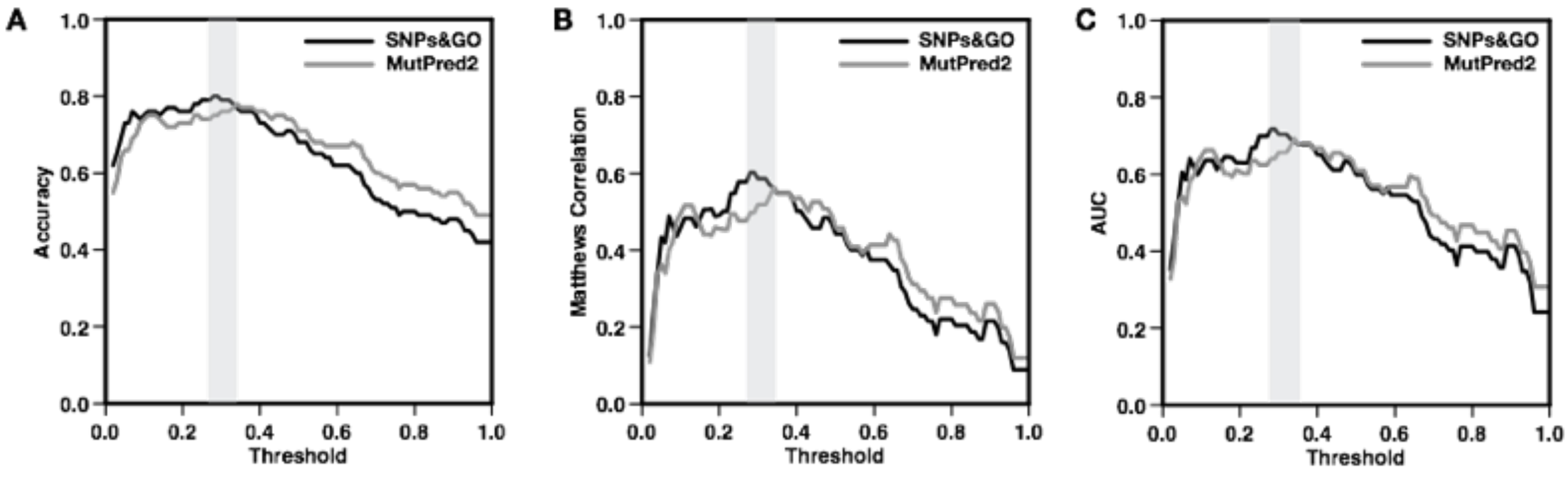
Challenge: Predict the effect of the 165 variants on NAGLU enzymatic activity.

The submitted prediction should be a numeric value ranging from 0 (no activity) to 1 (wild-type level of activity).

A posteriori evaluation

An evaluation of the performance shows that SNPs&GO reaches similar accuracy than the best method (MutPred2)

Method	Q2	AUC	MC	RMSE	$r_{Pearson}$	$r_{Spearman}$	$r_{KendallTau}$
MutPred2	0.780	0.850	0.565	0.30	0.595	0.619	0.443
SNPs&GO	0.800	0.854	0.603	0.33	0.575	0.616	0.445
SNPs&GO^{09}	0.750	0.749	0.499	0.46	0.477	0.495	0.409



Conclusions

- Evolutionary information is an important feature for the prediction of deleterious variants. The pathogenic variants tend to occur in conserved protein sites.

- Structural information encoded through the relative solvent accessibility and the structure environment improves the predictions of pathogenic variants.

- The implementation of meta-prediction based approach allows to select highly-accurate predictions.

- Nucleotide conservation is an important feature to predict the impact of SNVs also in noncoding regions.

Acknowledgments

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Biomolecules, Folding and Disease

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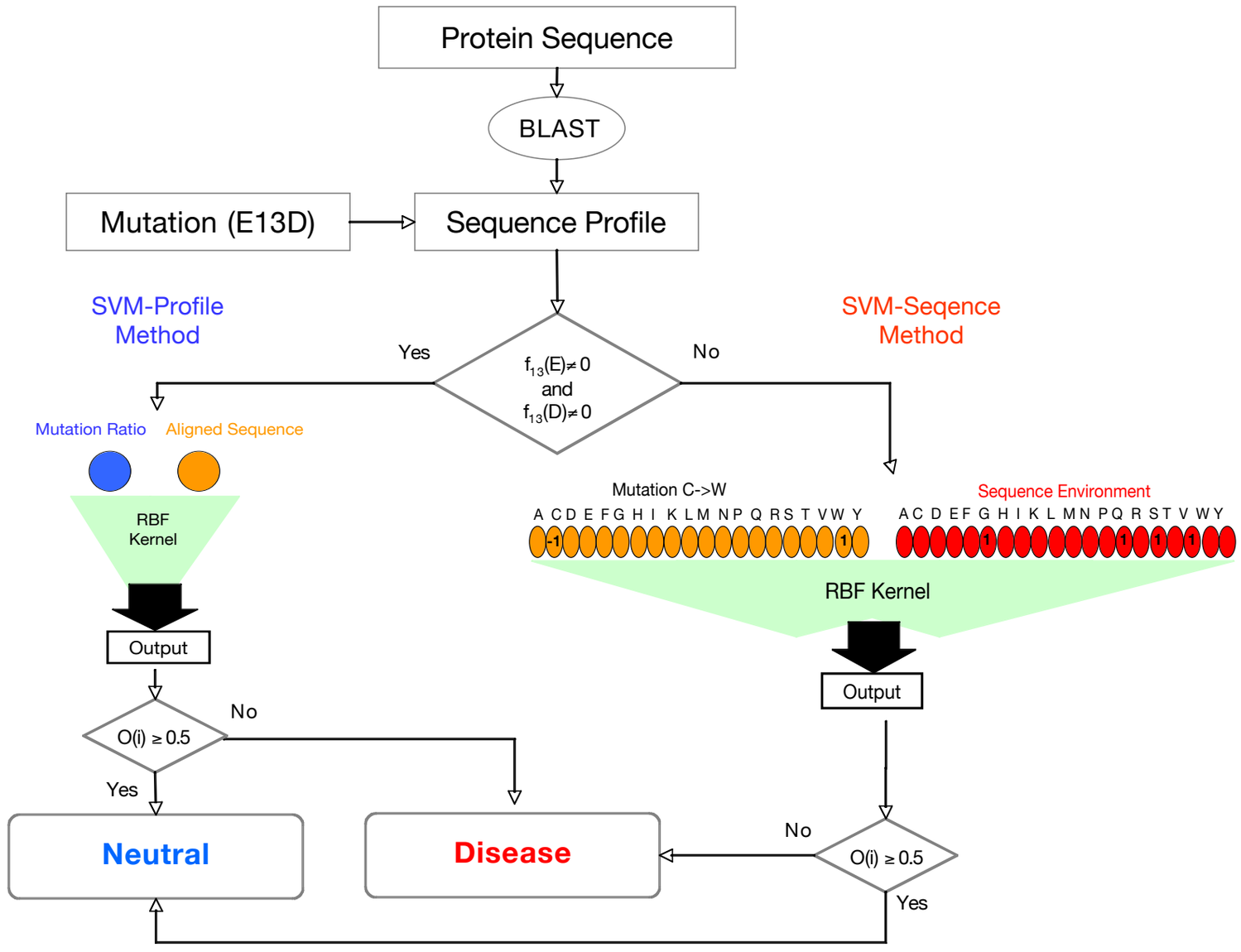
Hybrid method structure

Hybrid Method is based on a decision tree with SVM-Sequence coupled to SVM-Profile. Tested on more than 21,000 variants our method reaches 74% of accuracy and 0.46 correlation coefficient.



Capriotti et al. (2006) Bioinformatics, 22; 2729-2734.

Classification results

SVM–Sequence is more accurate in the prediction of disease related mutations and SVM-Profile is more accurate in the prediction of neutral polymorphism.
Both methods have the same Q2 level.

	Q2	P[D]	Q[D]	P[N]	Q[N]	C
SVM-Sequence	0.70	0.71	0.84	0.65	0.46	0.34
SVM-Profile	0.70	0.74	0.49	0.68	0.86	0.39
HybridMeth	0.74	0.80	0.76	0.65	0.70	0.46

D = Disease related N = Neutral

The Hybrid Method have higher accuracy than the previous two methods increasing the accuracy up to 74% and the correlation coefficient up to 0.46.

http://snps.biofold.org/phd-snp

Gene Ontology

The Gene Ontology project is a major bioinformatics initiative with the aim of standardizing the representation of gene and gene product attributes across species and databases. The project provides a controlled vocabulary of terms for describing gene product characteristics and gene product annotation data.

http://www.geneontology.org/

The ontology is represented by a direct acyclic graph covers three domains;

- cellular component, the parts of a cell or its extracellular environment;
- molecular function, the elemental activities of a gene product at the molecular level, such as binding or catalysis
- biological process, operations or sets of molecular events with a defined beginning and end, pertinent to the functioning of integrated living units: cells,tissues, organs and organisms.

The P16 challenge

CDKN2A is the most common, high penetrance, susceptibility gene identified to date in familial malignant melanoma. $p16^{INK4A}$ is one of the two oncosuppressor which promotes cell cycle arrest by inhibiting cyclin dependent kinase (CDK4/6).

Challenge: Evaluate how different variants of p16 protein impact its ability to block cell proliferation.

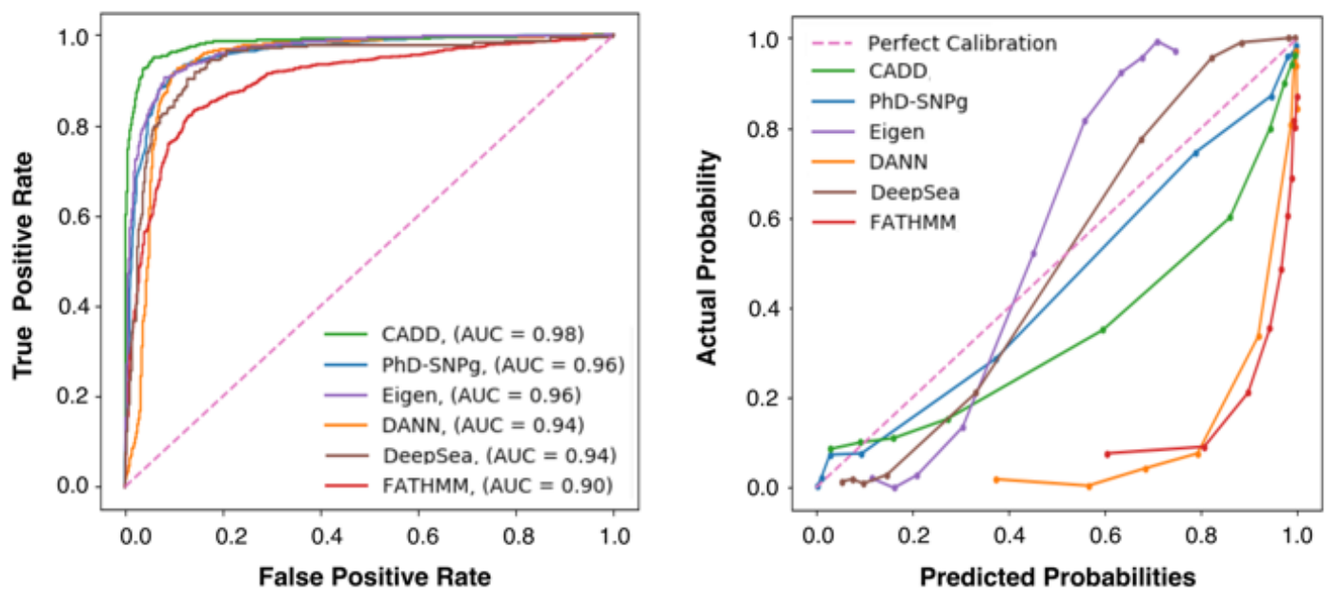
Provide a number between 50% that represent the normal proliferation rate of control cells and 100% the maximum proliferation rate in case cells.

Prediction calibration

The calibration refers to the correspondence between the probabilistic output of the method and the observed fraction of positive cases.

On ~2,000 newly annotated variants PhD-SNPg and CADD among the most accurate and calibrated methods with AUC > 0.96 and Brier Score < 0.07. Nevertheless CADD output needs to be transformed to be calibrated.

	BS_{All}	BS_{Coding}	$BS_{Noncoding}$
PhD-SNPg	0.07	0.10	0.03
CADD*	0.05	0.05	0.04



Benevenuta, Capriotti and Fariselli. (2020) Bioinformatics PMID: 33492342.

SNPs&GO prediction

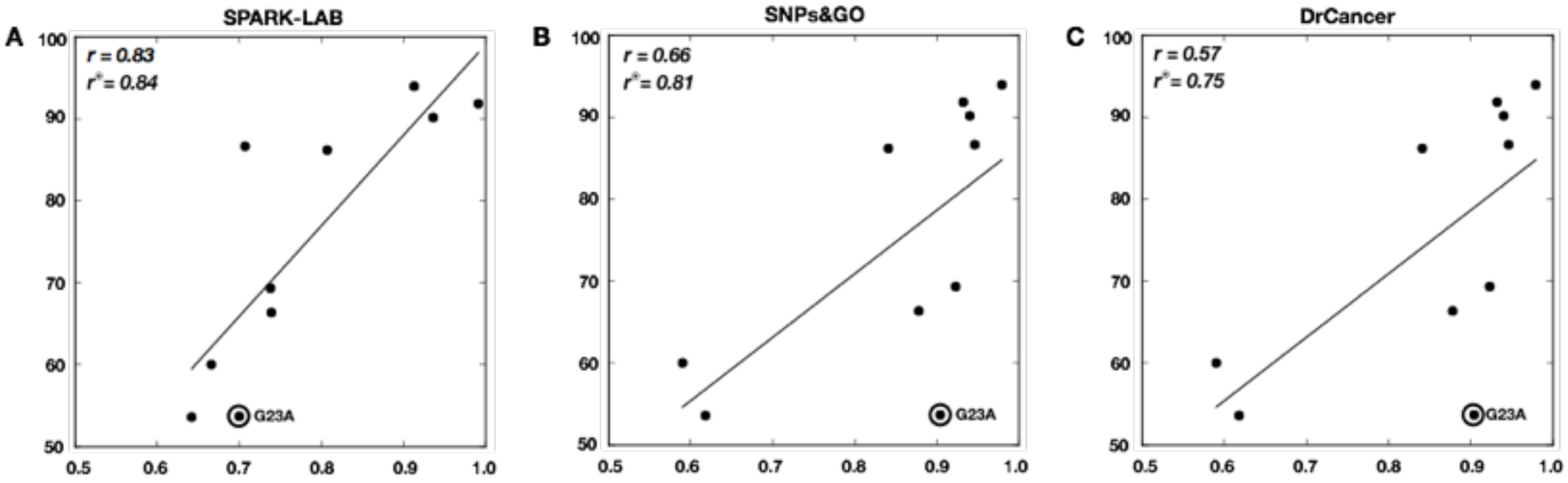
Proliferation rates predicted using the output of SNPs&GO without any optimization.

Variant	Prediction	Real	Δ	%WT	%MUT
G23R	0.932	0.918	0.014	84	0
G23S	0.923	0.693	0.230	84	1
G23V	0.940	0.901	0.039	84	0
G23A	0.904	0.537	0.367	84	2
G23C	0.946	0.866	0.080	84	0
G35E	0.590	0.600	0.010	12	14
G35W	0.841	0.862	0.021	12	0
G35R	0.618	0.537	0.081	12	4
L65P	0.878	0.664	0.214	15	1
L94P	0.979	0.939	0.040	56	0

P16 predictions

SNPs&GO resulted among the best methods for predicting the impact of P16INK4A variants on cell proliferation.

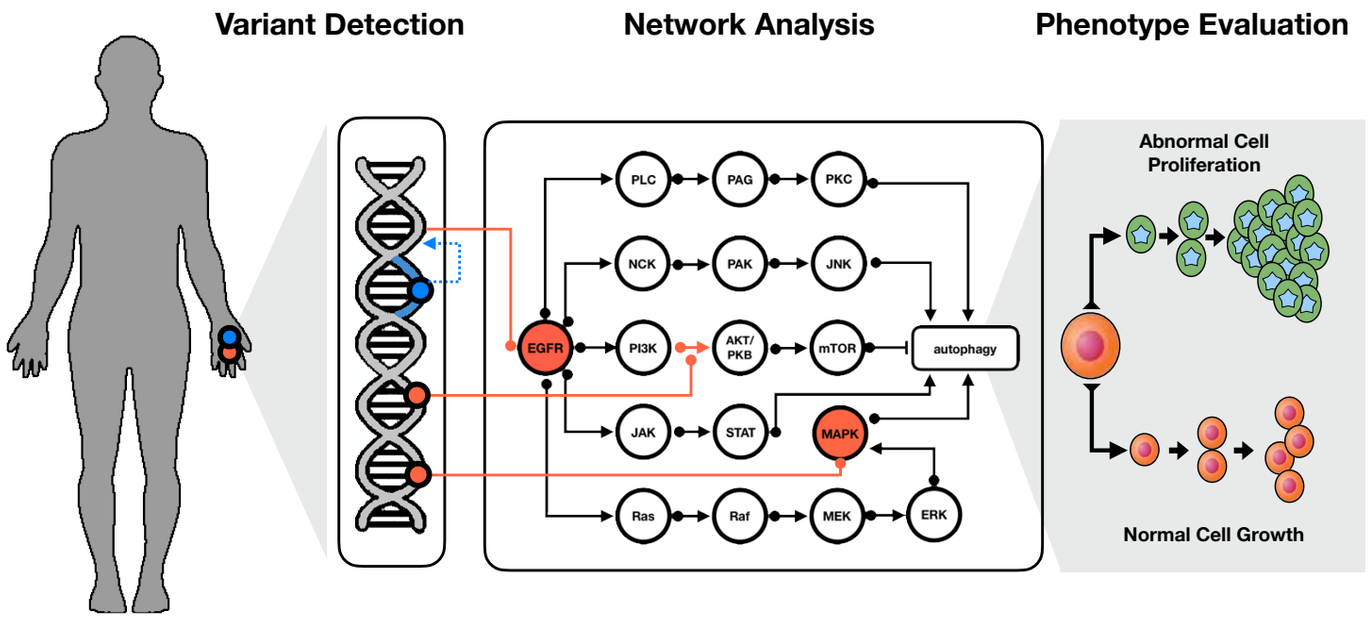
Method	Q2	AUC	MC	RMSE	$r_{Pearson}$	$r_{Spearman}$	$r_{KendallTau}$
SPARK-LAB	0.900	0.920	0.816	0.30	0.595	0.619	0.443
SNPs&GO	0.700	0.880	0.500	0.33	0.575	0.616	0.445
DrCancer	0.600	0.840	0.333	0.46	0.477	0.495	0.409

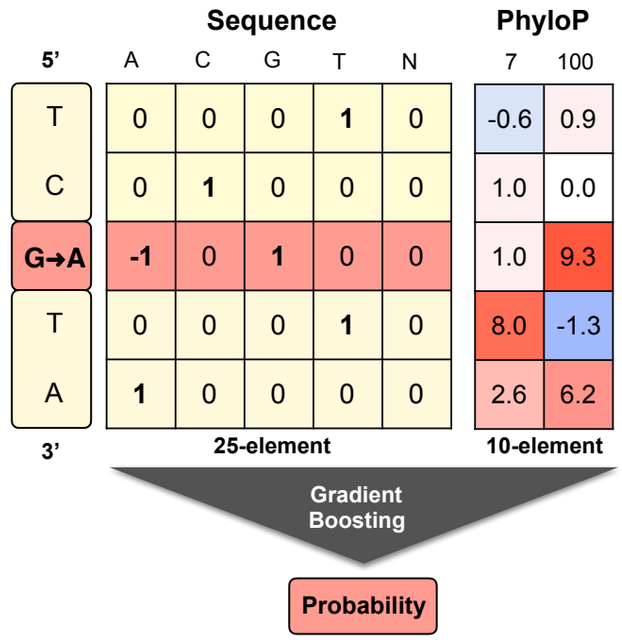


Capriotti et al. (2017) Human Mutations. PMID: 28102005.

PhD-SNPg

Variations in regulatory regions can perturb gene networks changing the topology or the edge weight of the biological network





PhD-SNPg implements a gradient-boosting algorithms that can run relying only on web resources

http://snps.biofold.org/phd-snpg

Capriotti and Fariselli. (2017) Nucleic Acids Res. 45: W247-W252. (IF 11.15)