Predicting the effect of protein variants

Laboratory of Bioinformatics I
Module 2

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http://biofold.org/

Biomolecules Folding and Disease

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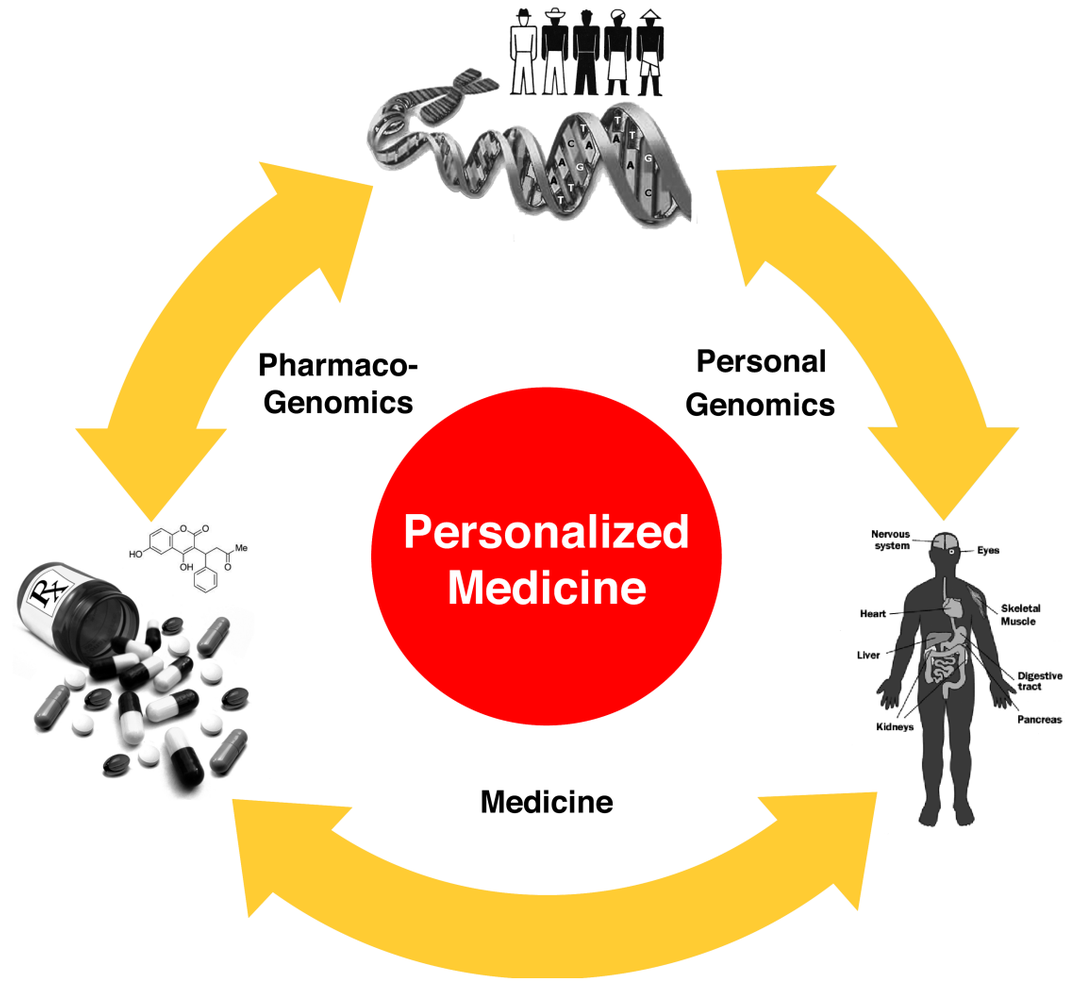
Personalized medicine

Currently direct to consumers company are performing genotype test on markers associated to genetic traits, and and soon full genome sequencing will cost about 1000$.

The future bioinformatics challenges for personalized medicine will be:

1. Processing Large-Scale Robust Genomic Data
2. Interpretation of the Functional Effect and the Impact of Genomic Variation
3. Integrating Systems and Data to Capture Complexity
4. Making it all clinically relevant



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

Single Nucleotide Variants

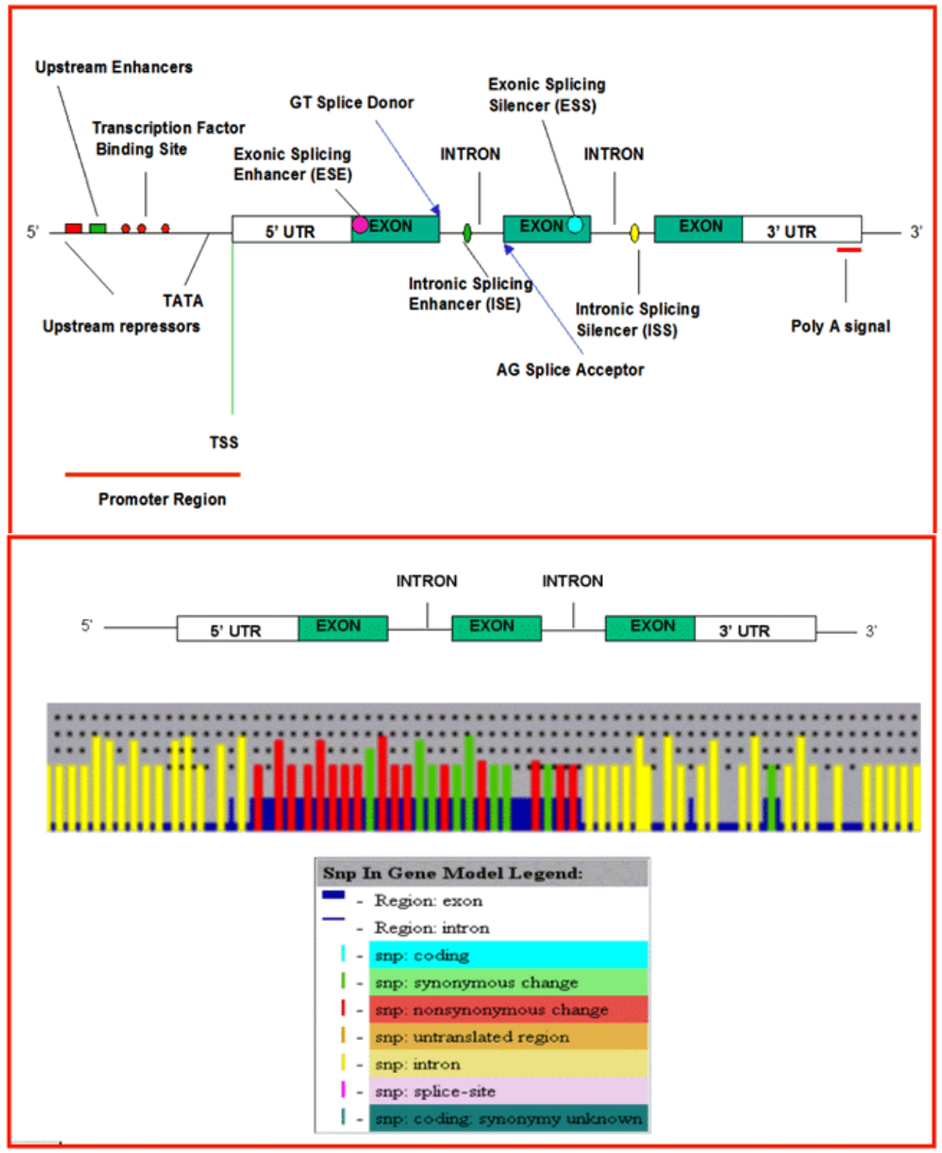
Single Nucleotide Variants (SNVs)
is a DNA sequence variation occurring when a single nucleotide A, T, C, or G in the genome differs between members of the species.
It is used to refer to Polymorphisms when the population frequency is ≥ 1%

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

Effects of variants

It is important to understand the functional effect of Single Nucleotide Polymorphisms (SNPs) that are very common type of variations, but also the impact rare variants which have allele frequencies below than 1%

Impact of coding variants

- Properties of amino acid residue substitution
- The evolutionary history of an amino acid position
- Sequence–function relationships
- Structure–function relationships

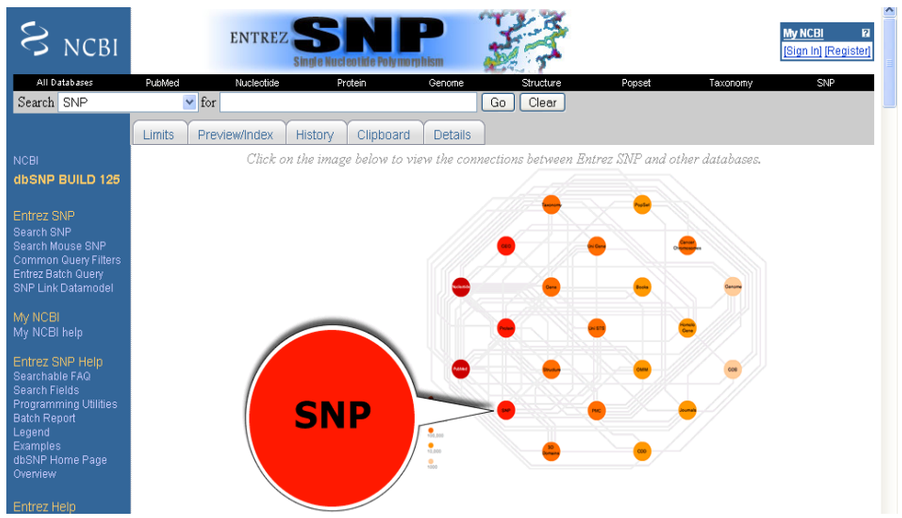
Impact of non-coding variants

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

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

SNVs and SAVs databases

dbSNP 147 (Apr 2016) @ NCBI



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

Single Nucleotide Variants

Homo sapiens	**100,815,862***
Bos taurus	100,170,652
Mus musculus	80,443,437

** Validated*

SwissVar (Jul 2016) @ ExPASy

SIB ExPASy

swissvar

http://www.expasy.ch/swissvar/

Single Amino acid Variants

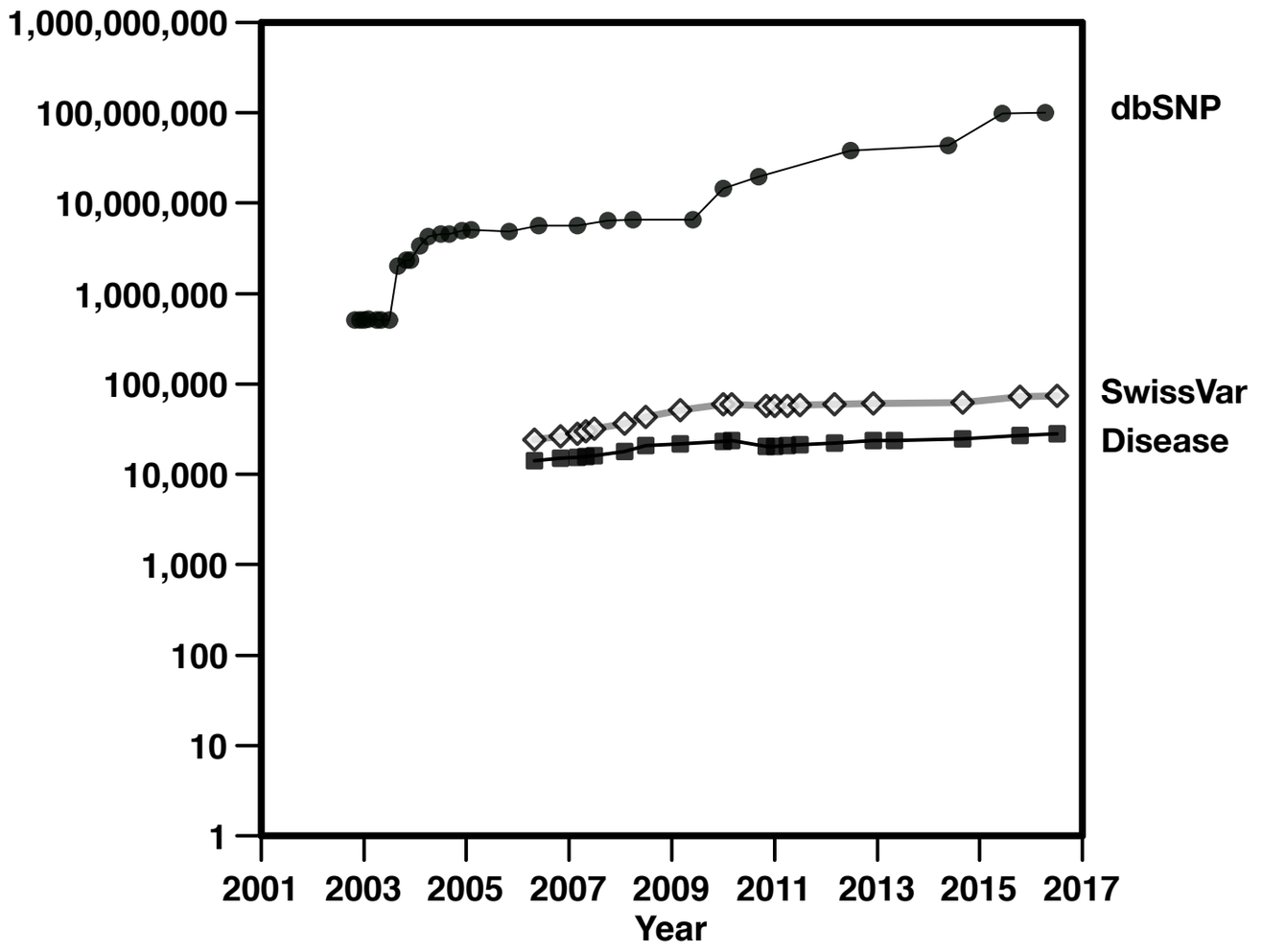
Homo sapiens	74,177
Disease	*28,239*
Polymorphisms	*38,934*

Sep 2016

SNVs and Disease

Single Nucleotide Variants (SNVs) are the most common type of genetic variations in human accounting for more than 90% of sequence differences (1000 Genome Project Consortium, 2012).

SNVs can also be responsible of genetic diseases (Ng and Henikoff, 2002; Bell, 2004).



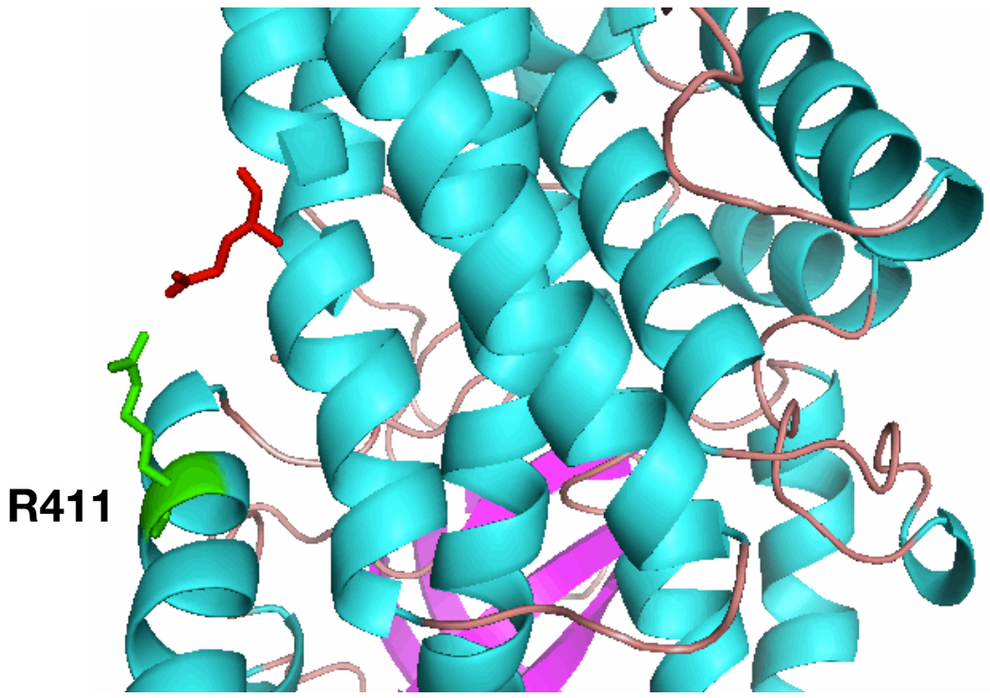
nonsynonymous SNVs

- disease-related SNVs → the mutations are related to a Mendelian pathologies
- neutral SNVs → the mutations do not compromise the organism's health

Sequence, Structure & Function

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

Nonsynonymous variants responsible for protein structural changes and cause loss of stability of the folded protein.
Mutation R411L removes the salt bridge stabilizing the structure of the IVD dehydrogenase.



What predictions?

Given the large amount of available mutations what can we predict?

Develop binary classifiers to predict the impact of mutations on:

- Protein Structure
- Protein Function
- Human Health

Structural changes upon mutation can be predicted using comparative modeling approaches.

Functional changes can be predicted from experimental data collected in PMD database (at http://www.genome.jp/dbget/)

Predicting the impact of mutation on human health is a more complex task that requires the integration of several source of information.

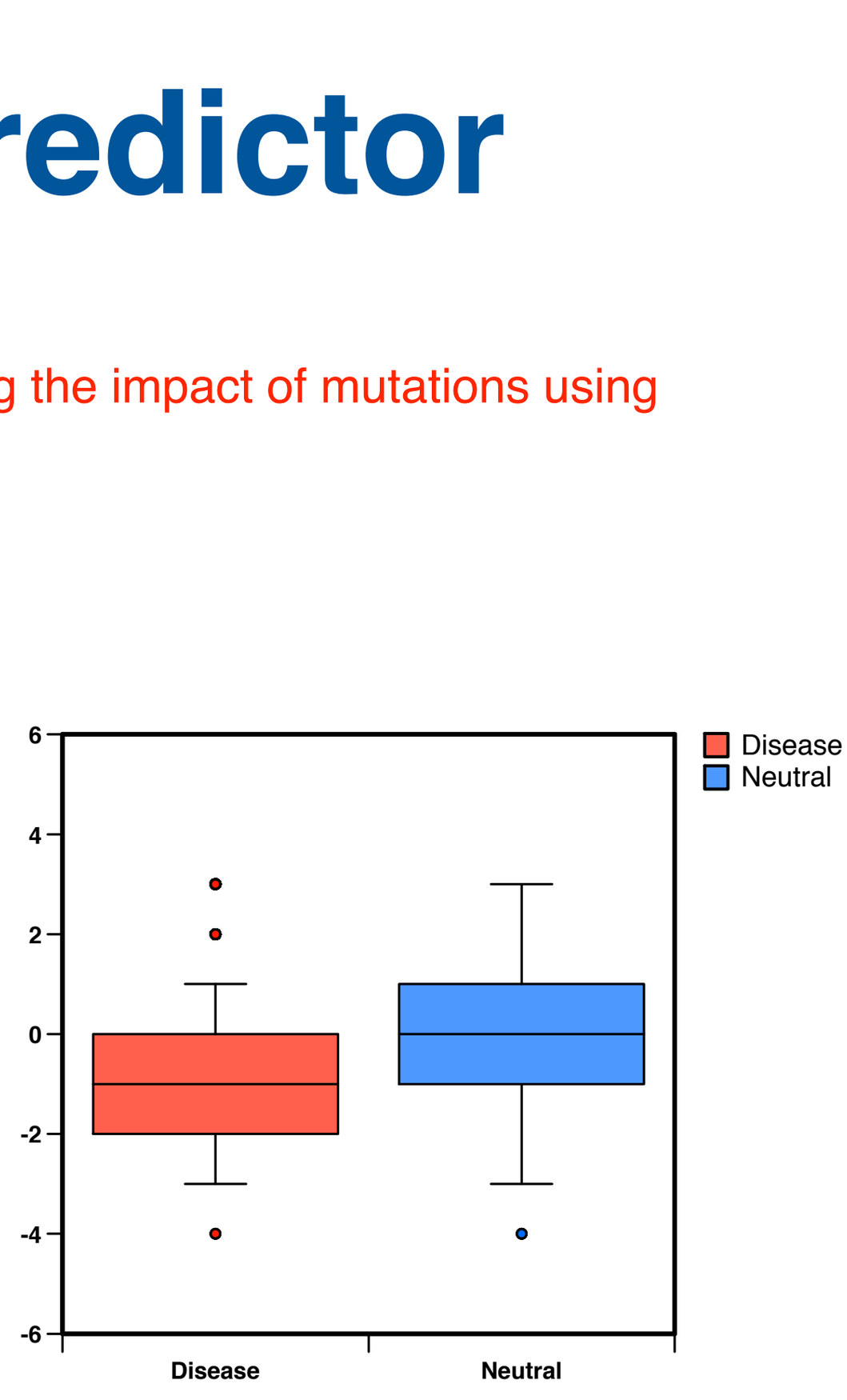
Simple Predictor

A simple method can be developed predicting the impact of mutations using BLOSUM62 substitution matrix.

BLOSUM62

	A	R	N	D	C	Q	E	G	H	I	L	K	M	F	P	S	T	W	Y	V	
A	5	-2	-2	-2	0	0	0	0	-2	-2	-3	-2	-1	-2	0	0	0	-2	-3	0	A
R		5	-2	-3	-3	0	-1	-2	0	-3	-4	1	-3	-3	-2	-2	0	0	-3	-4	R
N			5	0	0	0	-2	0	0	-4	-5	-2	-3	-3	-2	0	0	-2	-2	-5	N
D				5	-4	0	1	-1	0	-5	-6	-3	-4	-4	0	-2	-2	-2	-2	-5	D
C					8	-2	-3	-1	-1	0	-2	-3	0	-1	-1	1	0	0	-2	0	C
Q						5	2	0	0	-2	-4	0	-2	-3	0	0	0	0	-2	-3	Q
E							5	0	0	-3	-4	0	-3	-3	0	0	0	-2	-3	-3	E
G								6	0	-4	-5	-2	-3	-2	-2	0	0	0	-2	-3	G
H									6	-3	-4	0	-2	0	0	0	0	0	2	-2	H
I										4	0	-3	2	0	-2	-3	0	0	-3	2	I
L											4	-4	0	0	-3	-4	-3	0	-4	0	L
K												4	-2	-4	-1	-2	0	0	-3	-4	K
M													6	0	-3	-3	-2	0	-3	2	M
F														6	-3	-2	-2	2	2	0	F
P															7	0	0	-2	-3	0	P
S																4	2	-2	-2	-3	S
T																	5	-1	-3	0	T
W																		9	2	-1	W
Y																			7	-3	Y
V																				4	V

$$sim(i,j) = \log \frac{P(i,j)}{P(i)P(j)}$$

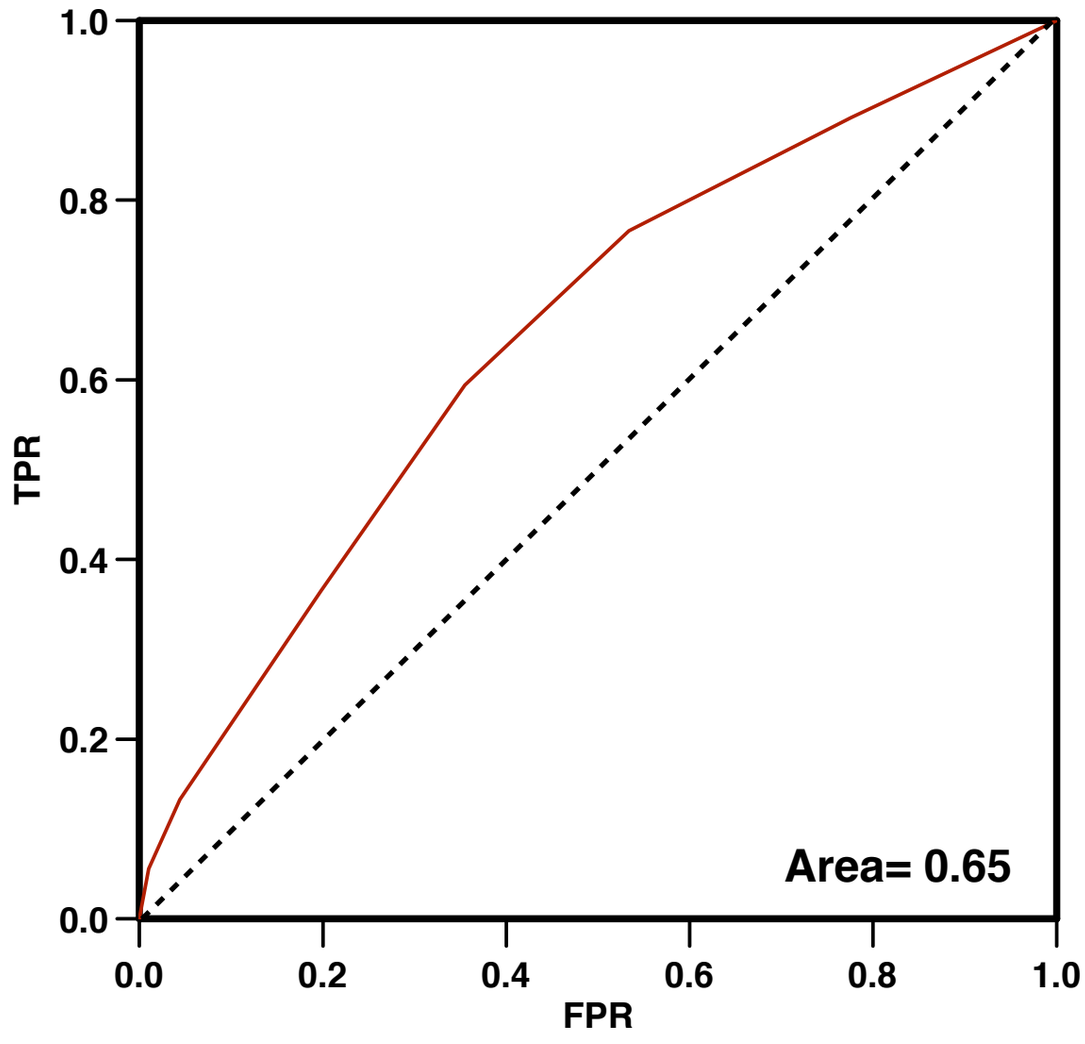


BLOSUM62 Predictions

It is possible to plot the ROC curve of the predictions moving BLOSUM62 threshold from -4 to 3.

We can calculate the Area Under the Curve and optimize the prediction threshold.

If we use a threshold equal to -1 the method result in 64% overall accuracy and 0.24 Matthews' correlation coefficient



	Q2	P[D]	S[D]	P[N]	S[N]	C
BLOSUM62	0.64	0.67	0.77	0.59	0.47	0.24

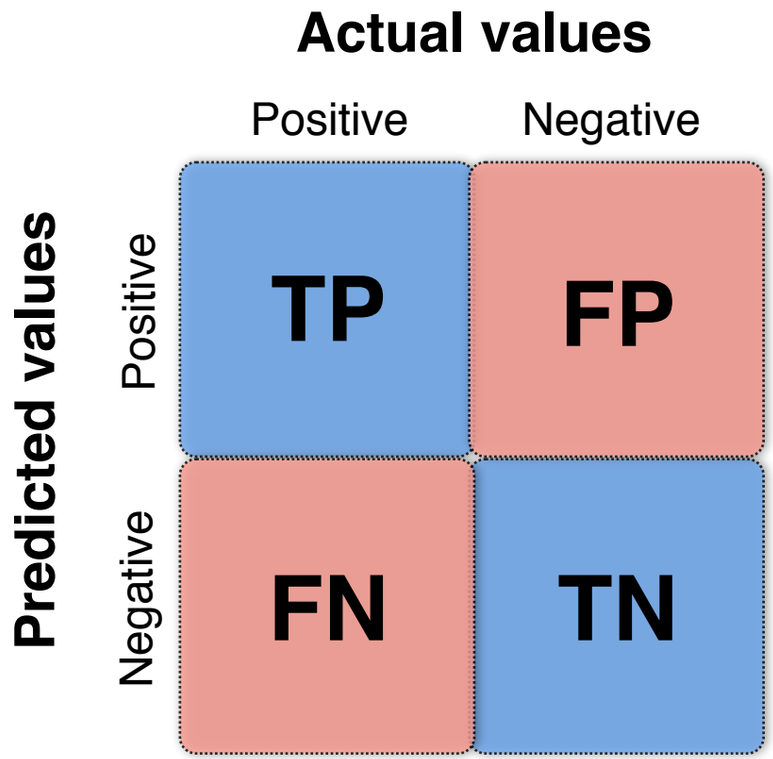
Accuracy measures

Overall Accuracy

$$Q2 = \frac{TP + TN}{TP + FN + TN + FP}$$

Sensitivity

$$S = \frac{TP}{TP + FN}$$



Precision

$$P = \frac{TP}{TP + FP}$$

Correlation

$$C = \frac{TP \times TN - FP \times FN}{\sqrt{(TP + FP) \times (TP + FN) \times (TN + FP) \times (TN + FN)}}$$

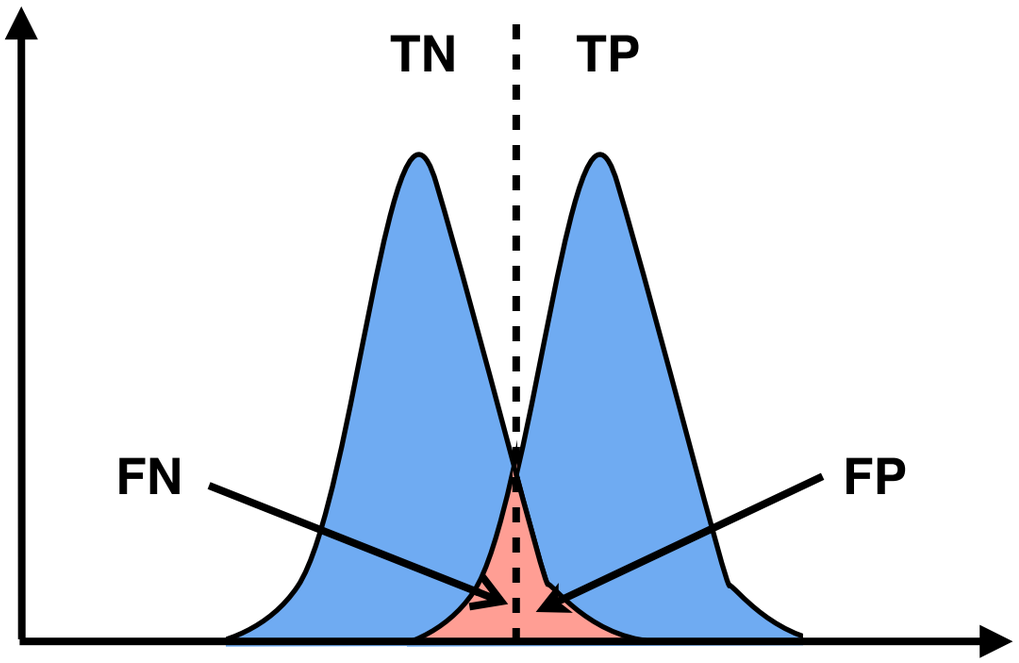
Receiving Operator Curve

True Positive Rate

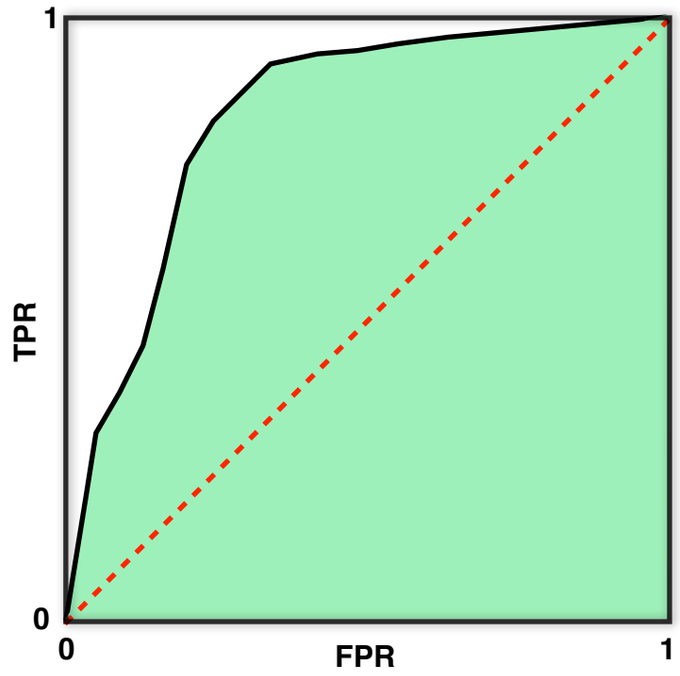
$$TPR = \frac{TP}{TP + FN}$$

False Positive Rate

$$FPR = \frac{FP}{FP + TN}$$



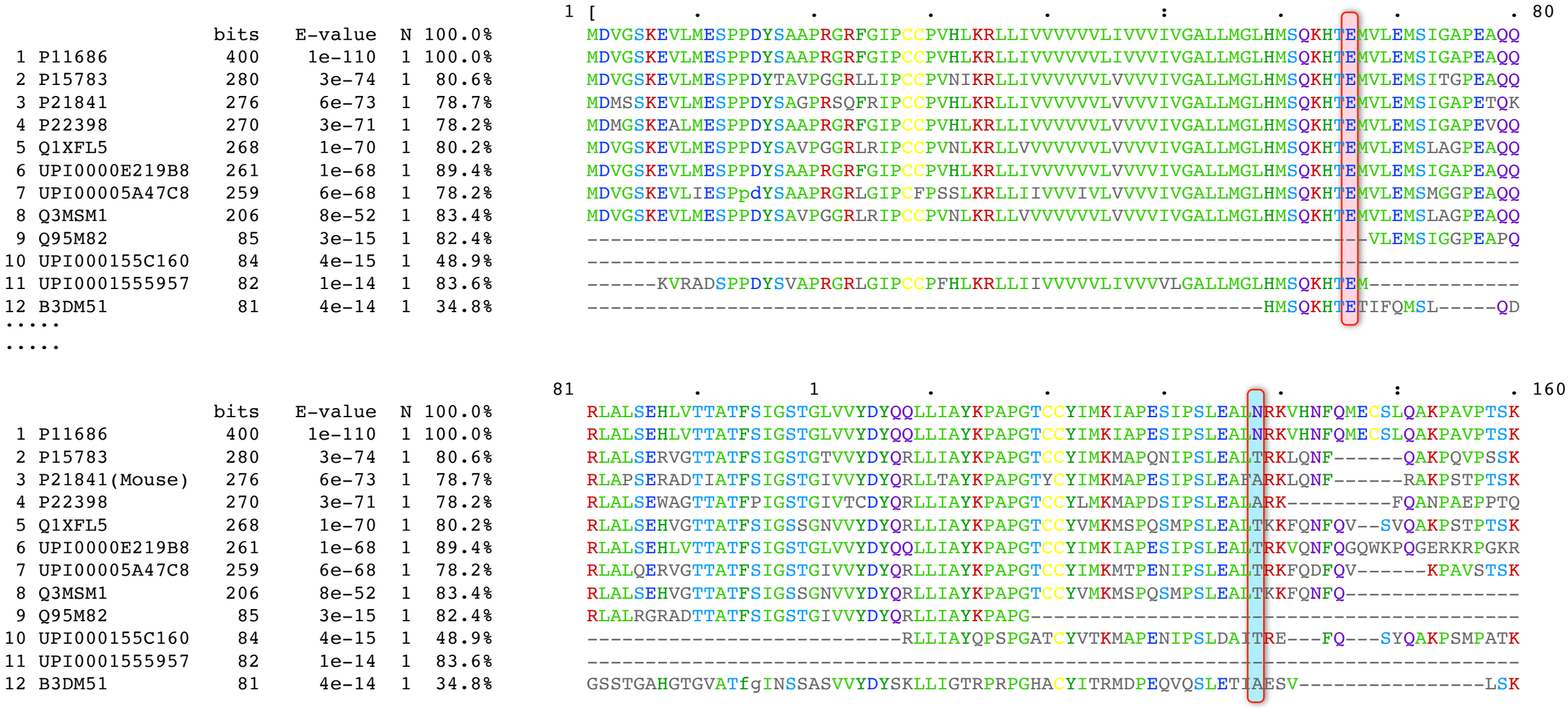
The Area Under the ROC Curve (AUC) is an accuracy measure that is 0.5 for completely random predictors and close to 1.0 for highly accurate predictors.



Baldi et al. (2000) Bioinformatics, 16:412-424

Conserved or not?

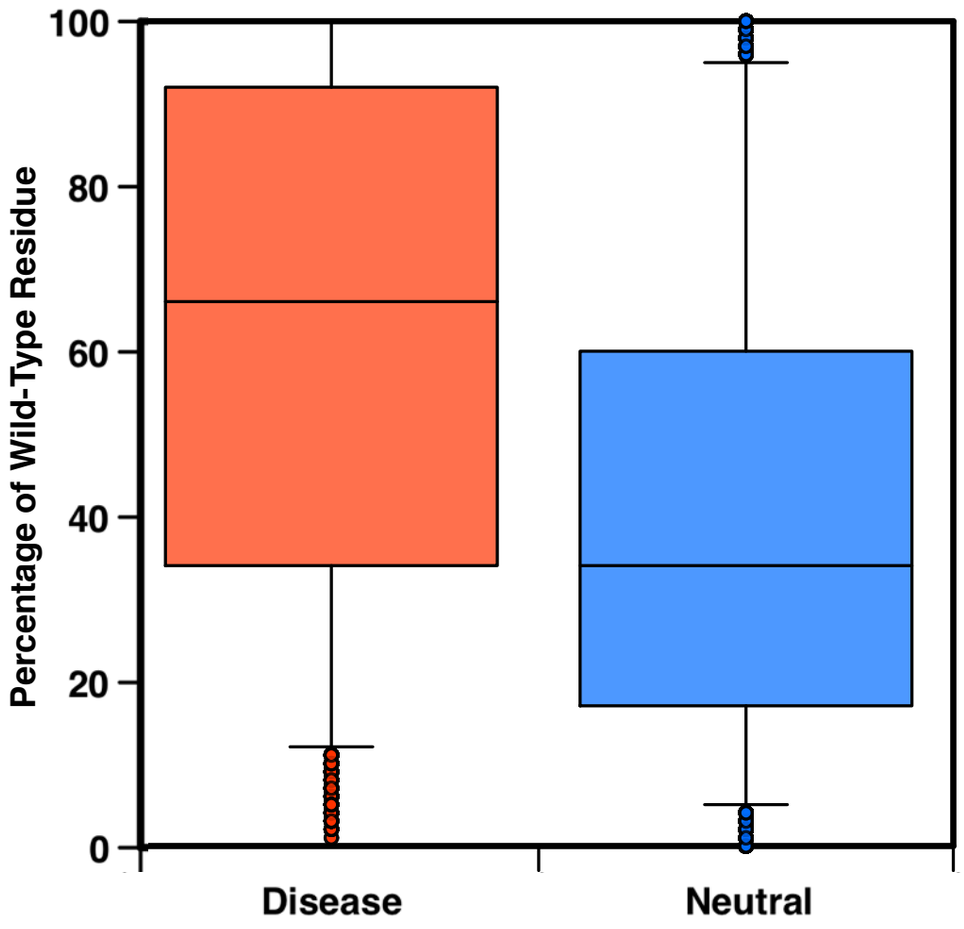
In positions 66 the Glutamic acid is highly conserved Asparagine in position 138 is mutated Threonine or Alanine



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 frequency distributions of the wild-type residues for disease-related and neutral variants are significantly different (KS p-value=0).



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

Machine learning

- Computational approach to build models based on the analysis of empirical data.

- Machine learning algorithms are suitable to address problems for which analytic solution does not exists and large amount of data are available.

- They are implemented selecting a representative set of data that are used in a training step and then validated on a test set with data *“not seen”* during the training.

- Most popular machine learning approaches are in computational biology are Neural Networks, Support Vector Machines and Random Forest.

Binary classifiers

- Support Vector Machine (SVM): Maps positive and negative training examples to a high-dimensional space in which they can be distinguished from each other.

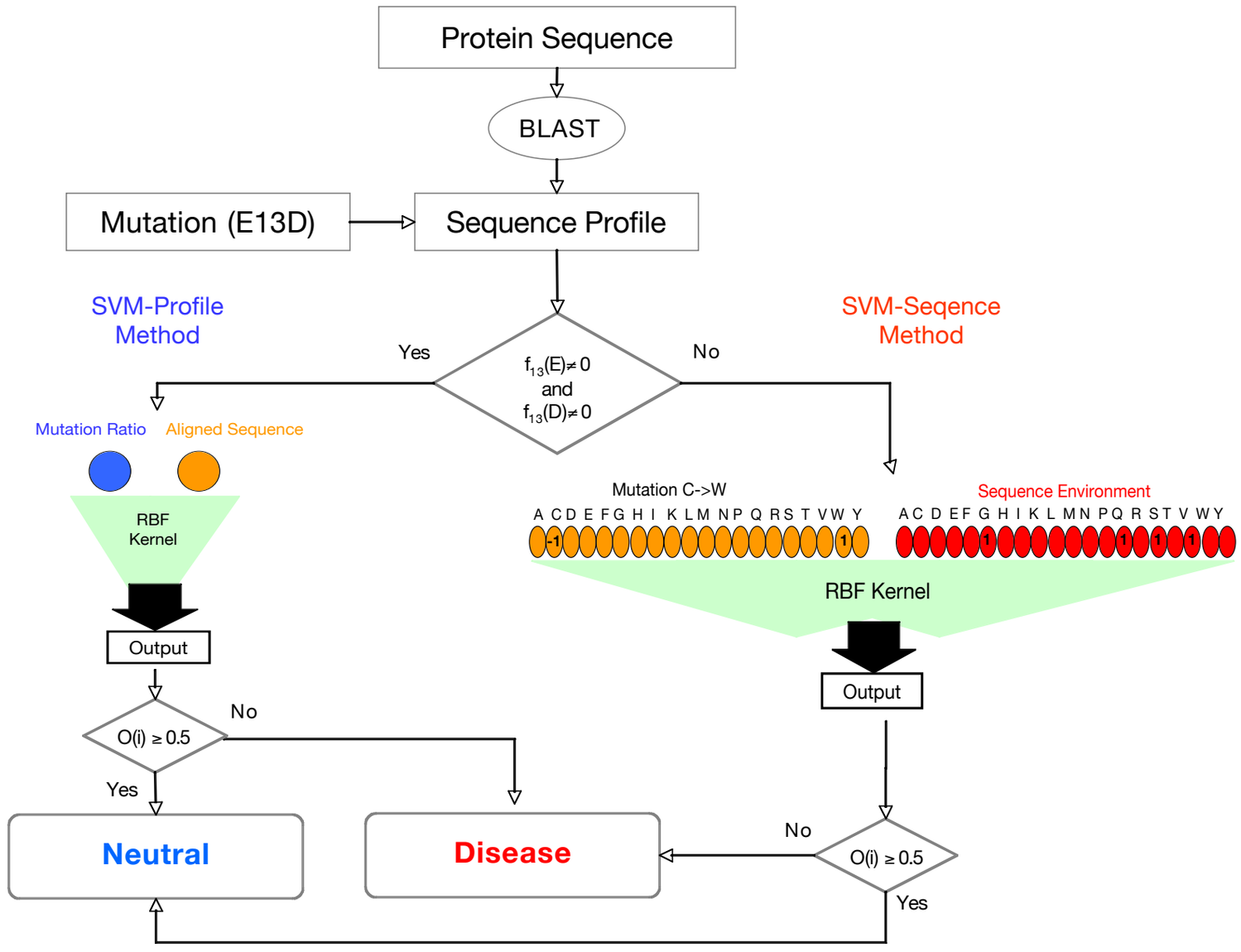
- Artificial Neural Network (ANN): multi-layer network of nodes, including input features, outputs, and one or more hidden layers. Weights of input and output edges connecting nodes are adjusted to maximize prediction accuracy.

- Random Forest (RF): Trains an “ensemble” of decision trees to distinguish positive from negative training examples, utilizing a random set of input features.

- Naïve Bayes Classifiers: Probabilistic classifier that treats each feature as independent of the others; parameters are adjusted to maximize the probability of impact for positive examples and minimize probability for negative examples.

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

Selective pressure

In genetics, the Ka/Ks ratio is an indicator of selective pressure acting on a protein-coding gene.

It is calculated as the ratio of the number of nonsynonymous substitutions per non-synonymous site (Ka), to the number of synonymous substitutions per synonymous site (Ks), in a given period of time.

Homologous genes with:

- Ka/Ks ratio >> 1 (positive selection): mutations must be advantageous.
- Ka/Ks ratio ~ 1 (neutral selection): advantageous ~ disadvantageous
- Ka/Ks ratio << 0 (negative selection): mutations are disadvantageous

The ratio, also known as ω or dN/dS, can be calculated at gene and site levels.

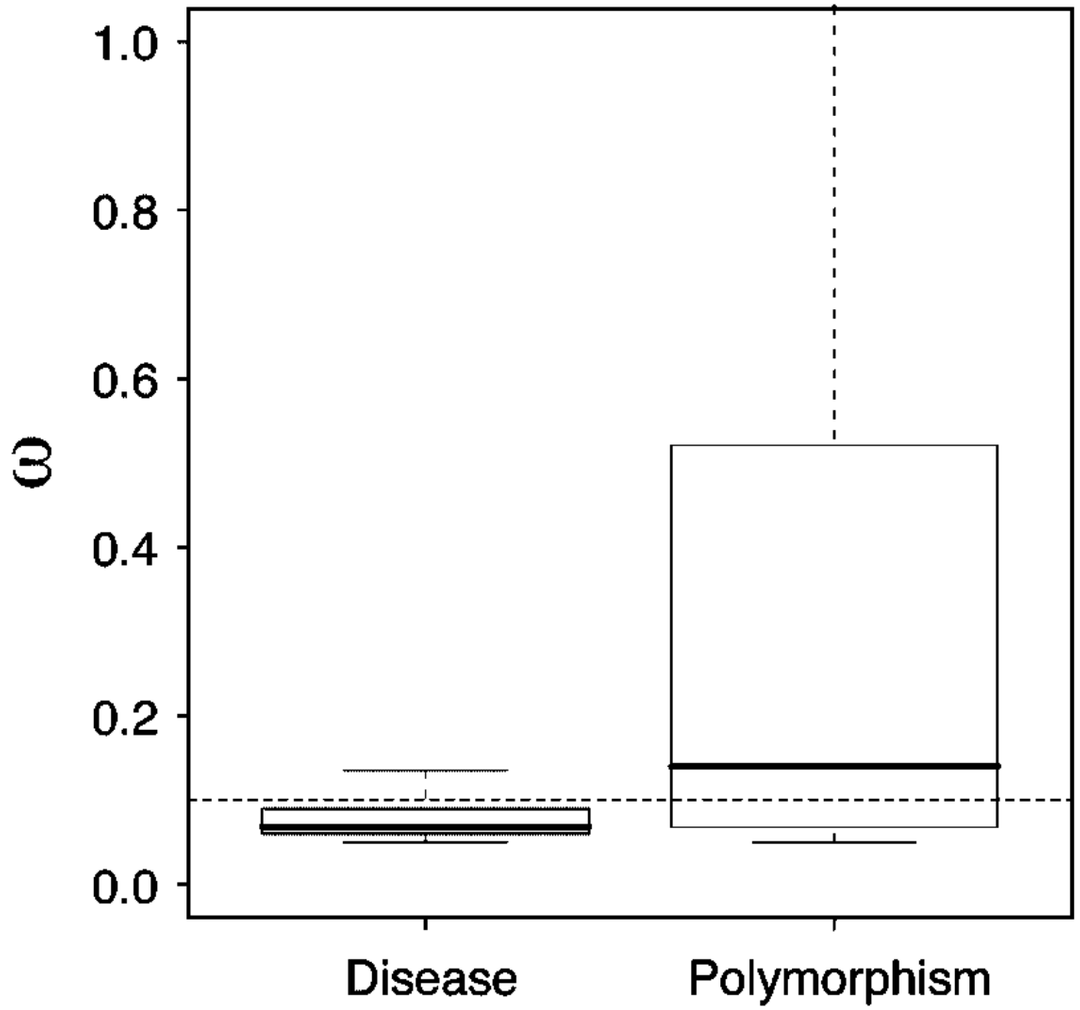
from Wikipedia

The omega values

In a previous work performed on 40 human disease genes, has been demonstrated that residues evolving under strong selective pressures (ω<0.1) are significantly associated with human disease (Arbiza et al. JMB, 2006).

We carried out a similar analysis on the dataset extracted from SwissProt and we found a statistically significant association between high selective pressures and disease in contrast to low selective pressures and neutral polymorphic variants in human.

$$\omega = \frac{dN}{dS}$$

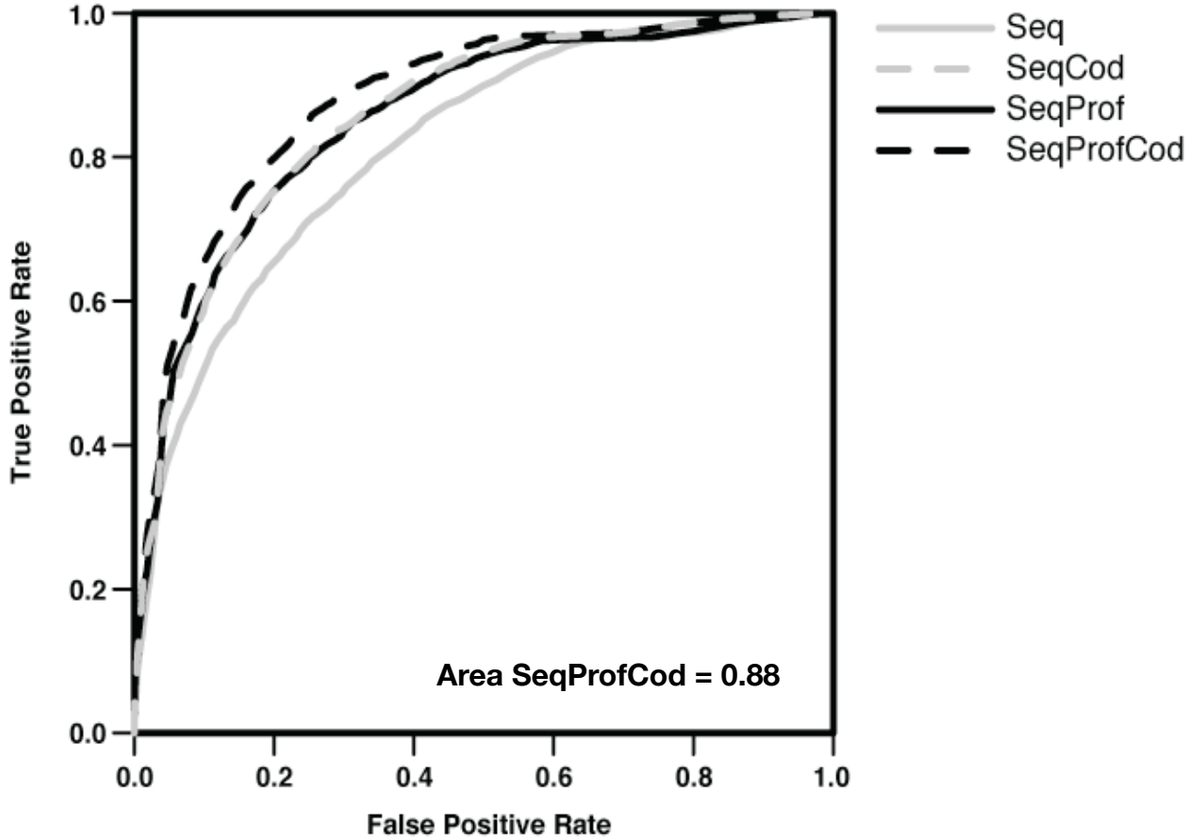


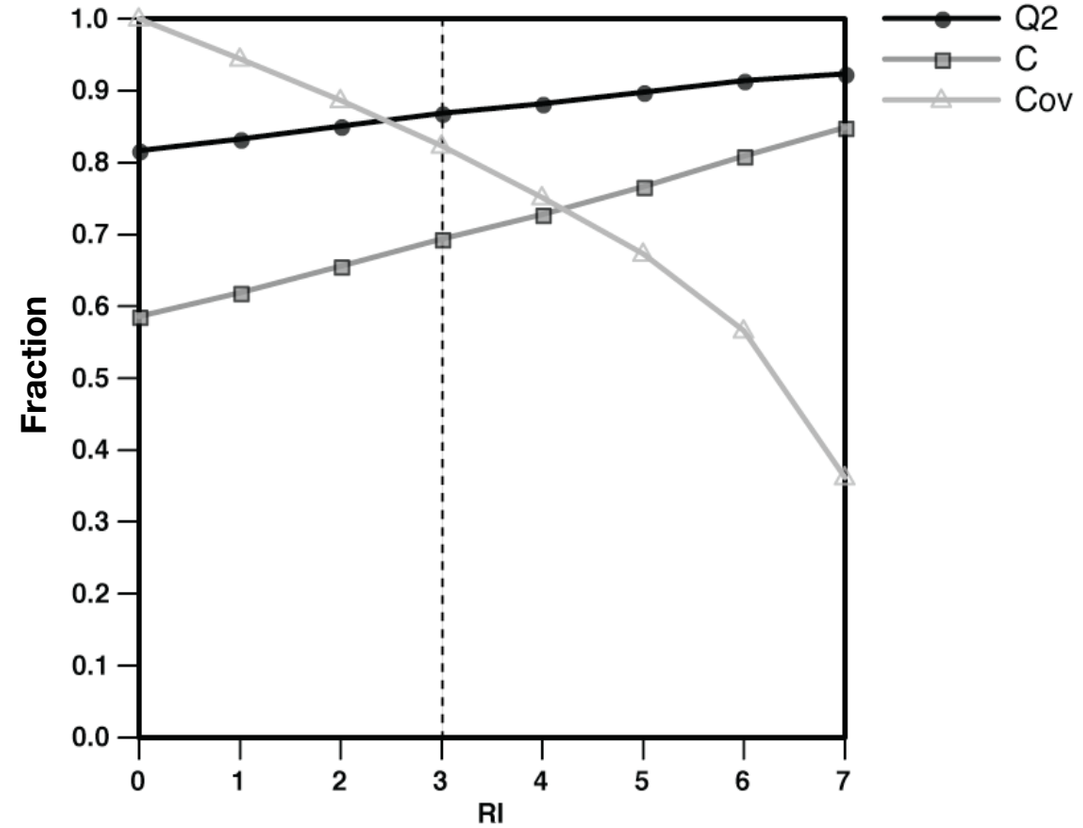
Capriotti et al. (2008) Human Mutation, 29: 198-204.

Omega-based method

SeqProfCod has higher accuracy than the previous two methods increasing the accuracy up to 82% and the correlation coefficient to 0.59.

	Q2	P[D]	Q[D]	P[N]	Q[N]	C
SeqProfCod	0.82	0.88	0.84	0.68	0.76	0.59





Q2: Overall Accuracy **C:** Correlation Coefficient **DB:** Fraction of database that are predicted with a reliability ≥ the given threshold

Gene Ontology

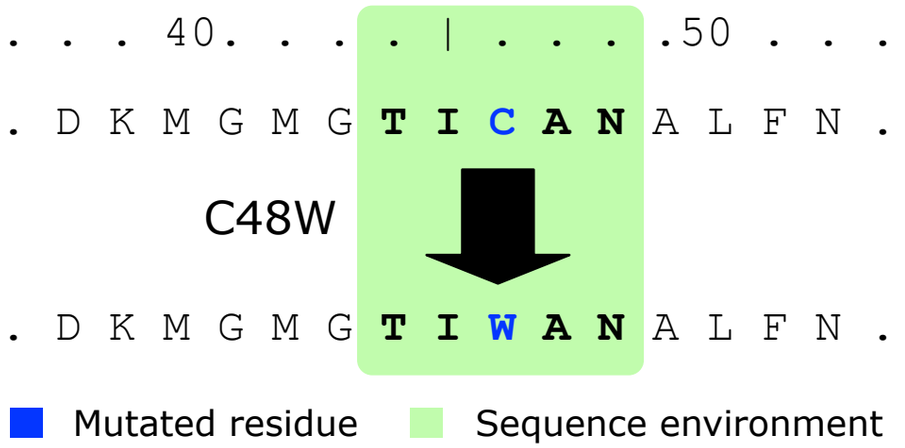
The Gene Ontology project is a major bioinformatics initiative with the aim of standardizing the representation of gene and gene product attributes across species and databases. The project provides a controlled vocabulary of terms for describing gene product characteristics and gene product annotation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

http://www.geneontology.org/

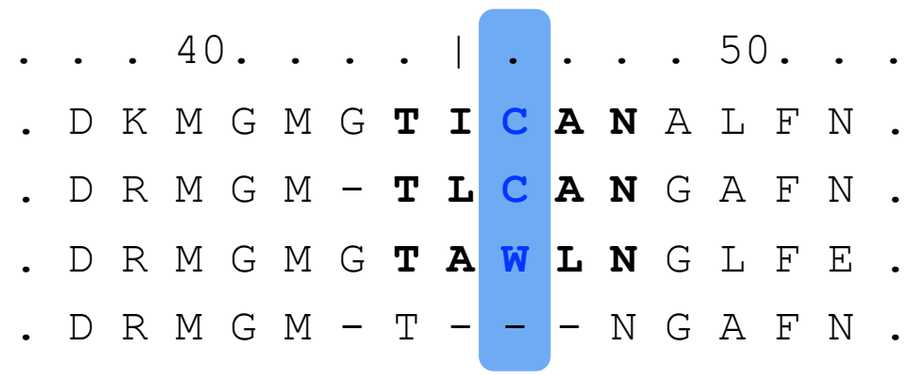
The ontology is represented by a direct acyclic graph covers three domains;

- 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.

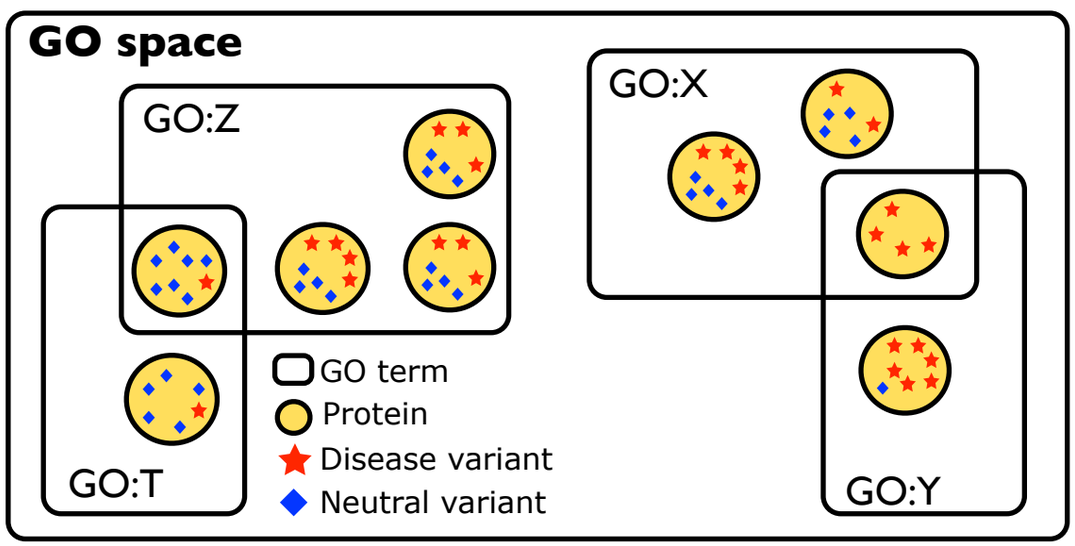
Prediction features



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



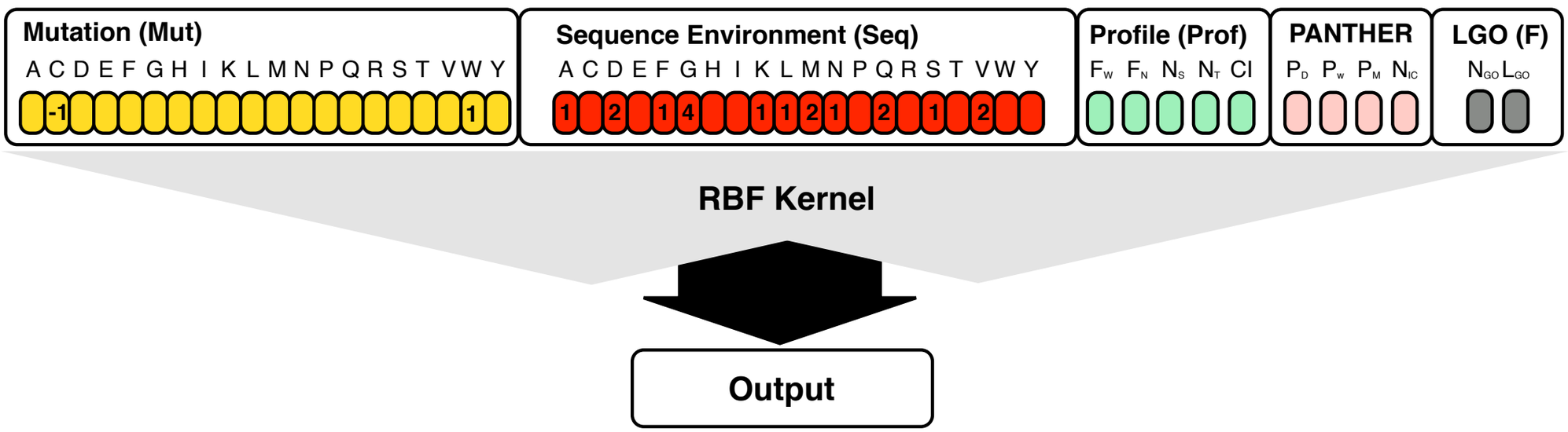
Protein sequence profile information derived from a multiple sequence alignment. It is encoded in a 5 elements vector corresponding to different features general and local features



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 disease-related and neutral 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 = Disease related N = Neutral

DB= 33672 nsSNVs

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

SwissVar data

SwissVar (October 2009)

- Disease variants: 22,771
- Neutral variants: 34,258
- Unclassified variants: 2,269
- **Total: 59,298**

- Disease-related mutations not clearly annotated are removed.

- Mutations related to more than one disease are considered only once.

Training set

After this filter we collected 17,993 Disease mutations from 1,424 proteins that are balanced with the same number of neutral polymorphisms.

Protein structure data

The mapping of SwissVar mutations data on the structures available on the PDB is a difficult task. The main problems for this task are:

- incomplete PDB structures
- differences between Swiss-Prot protein sequence and PDB sequence
- different residue numeration

The mapping procedure is performed using a pre-filtered list of correspondences between Swiss-Prot and PDB.

All Swiss-Prot/PDB pairs in the list are aligned using BLAST. To have a good overlap between sequence and structure I filtered the list of alignments removing those:

- with ≥ 1 gaps
- sequence identity < 100%
- shorter than 40 residues

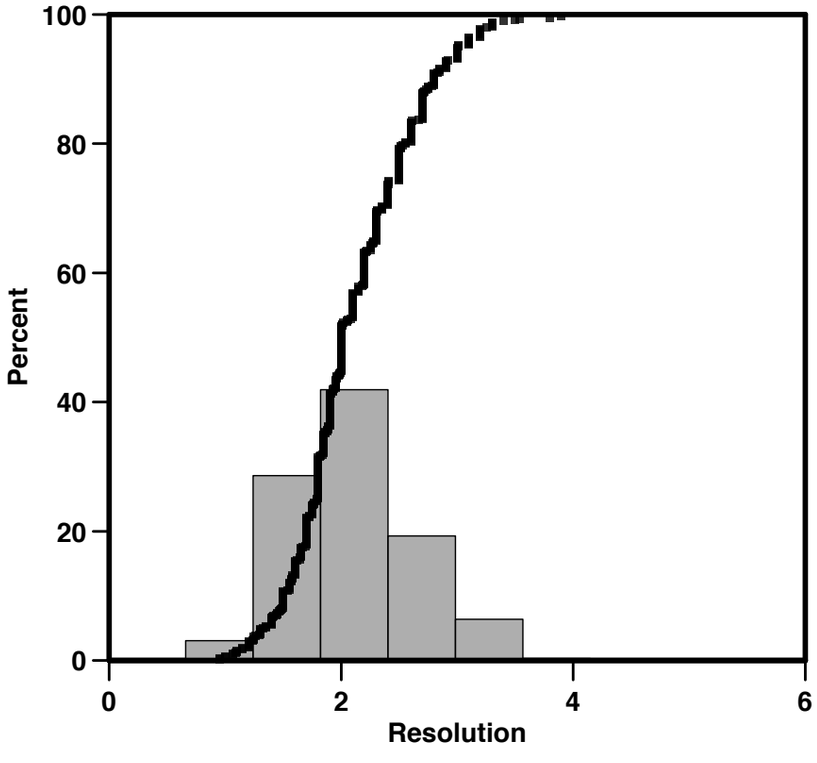
If one mutation maps on more than one PDB the one with lower resolution is selected

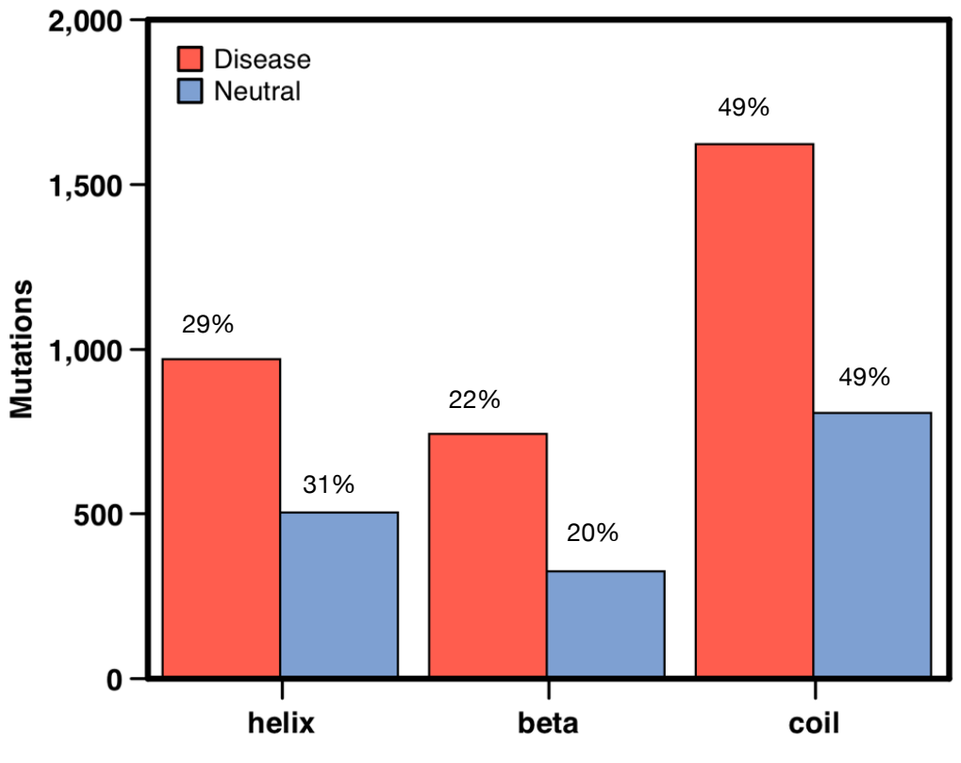
3D Structure Dataset

After the mapping procedure the final dataset of mutations with known 3D structure is composed by

- Disease variants: 3,342
- Neutral variants: 1,644
- **Total: 4,986**

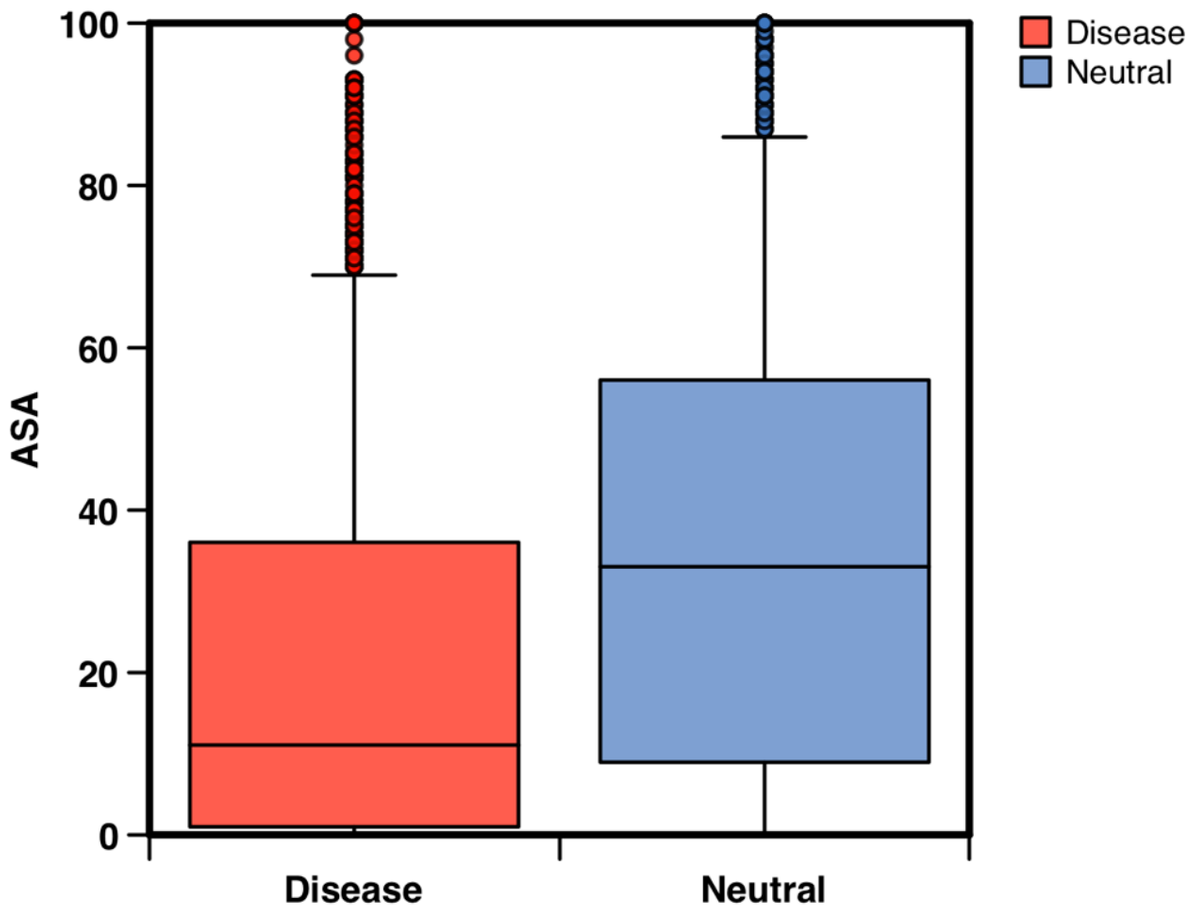
from 784 chains from 770 structures (584 X-ray, 92 NMR and 94 models).





Structure environment

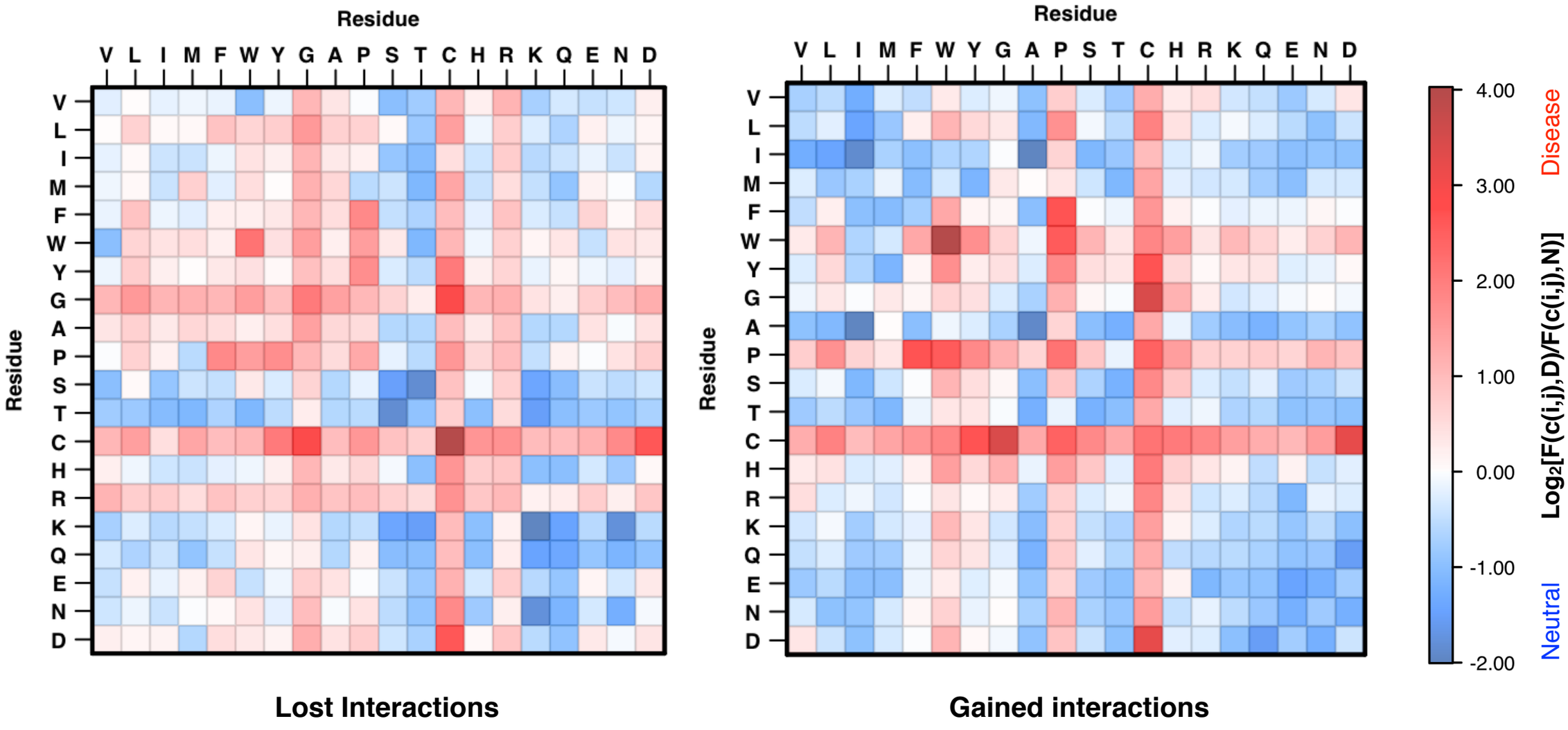
There is a significant difference (p-value KS < 0.001) between the distributions of the relative Accessible Solvent Area for disease-related and neutral variants.
Their mean values are respectively 20.6 and 35.7.



Analysis of the 3D interactions

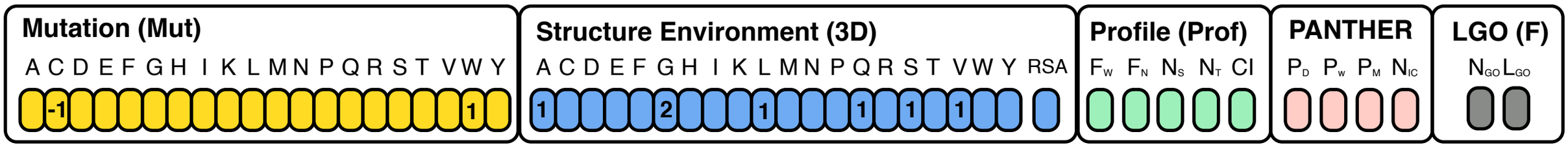
Using the whole set of SAVs with known structure, we calculate the log odd score of the ratio between the frequencies of the interaction between residue i and j for disease-related and neural variants.

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



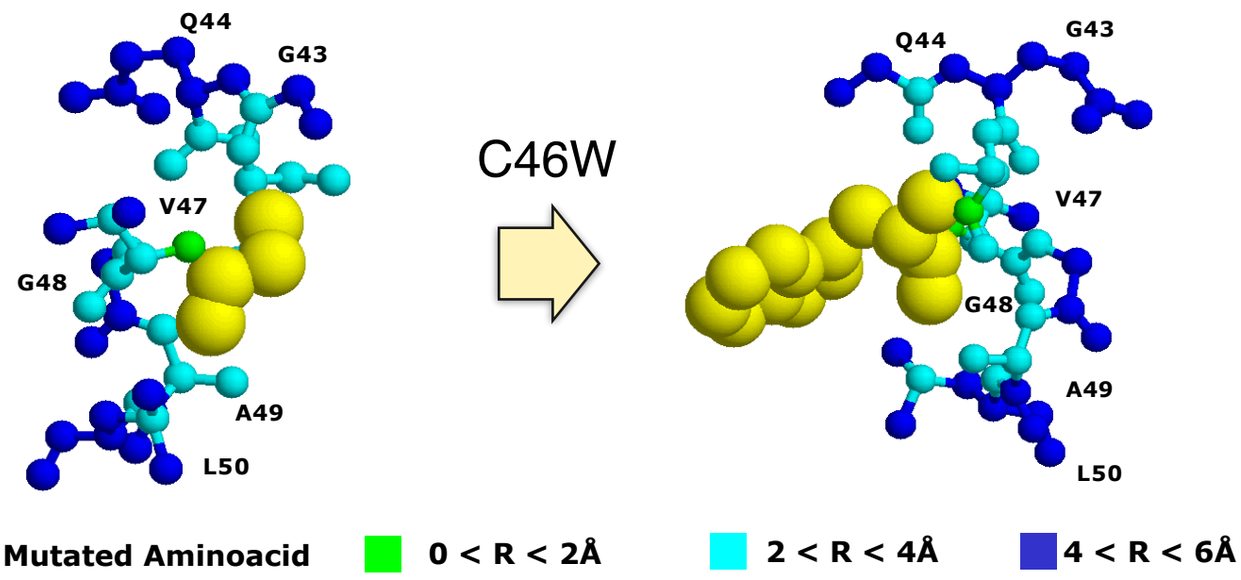
The structure-based method

The method takes in to account 5 different types of information encoded in a 52 elements vector. The input features are: mutation data; structure environment, sequence profile and functional score based on GO terms.



RBF Kernel

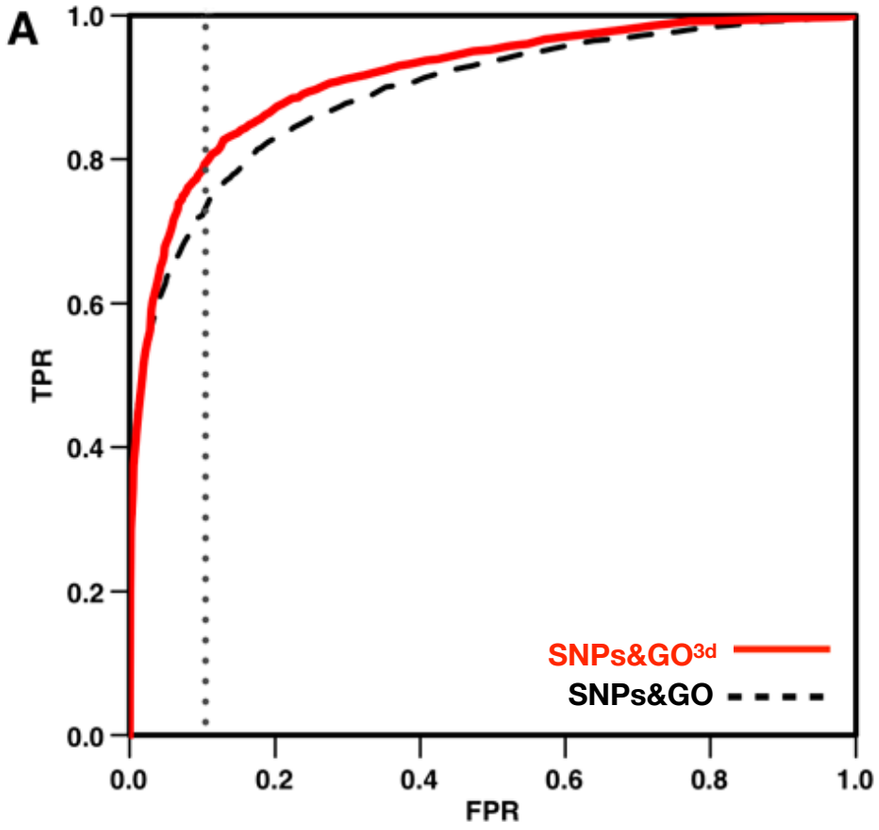
Output

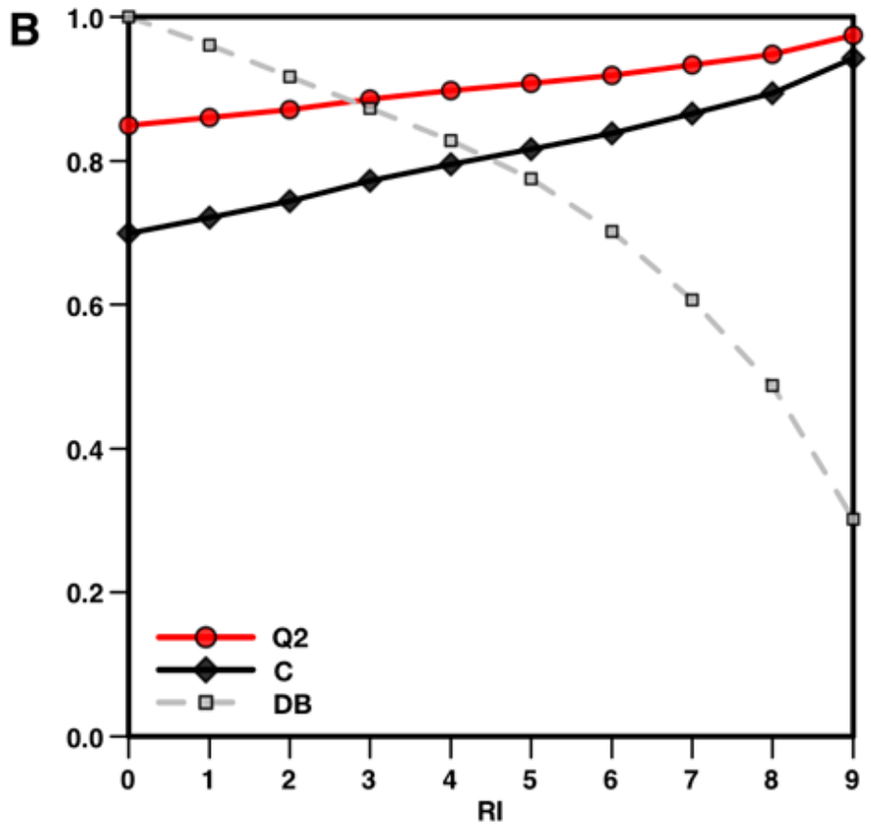


Sequence vs structure

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 FPR are accepted the TPR increases of 7%.

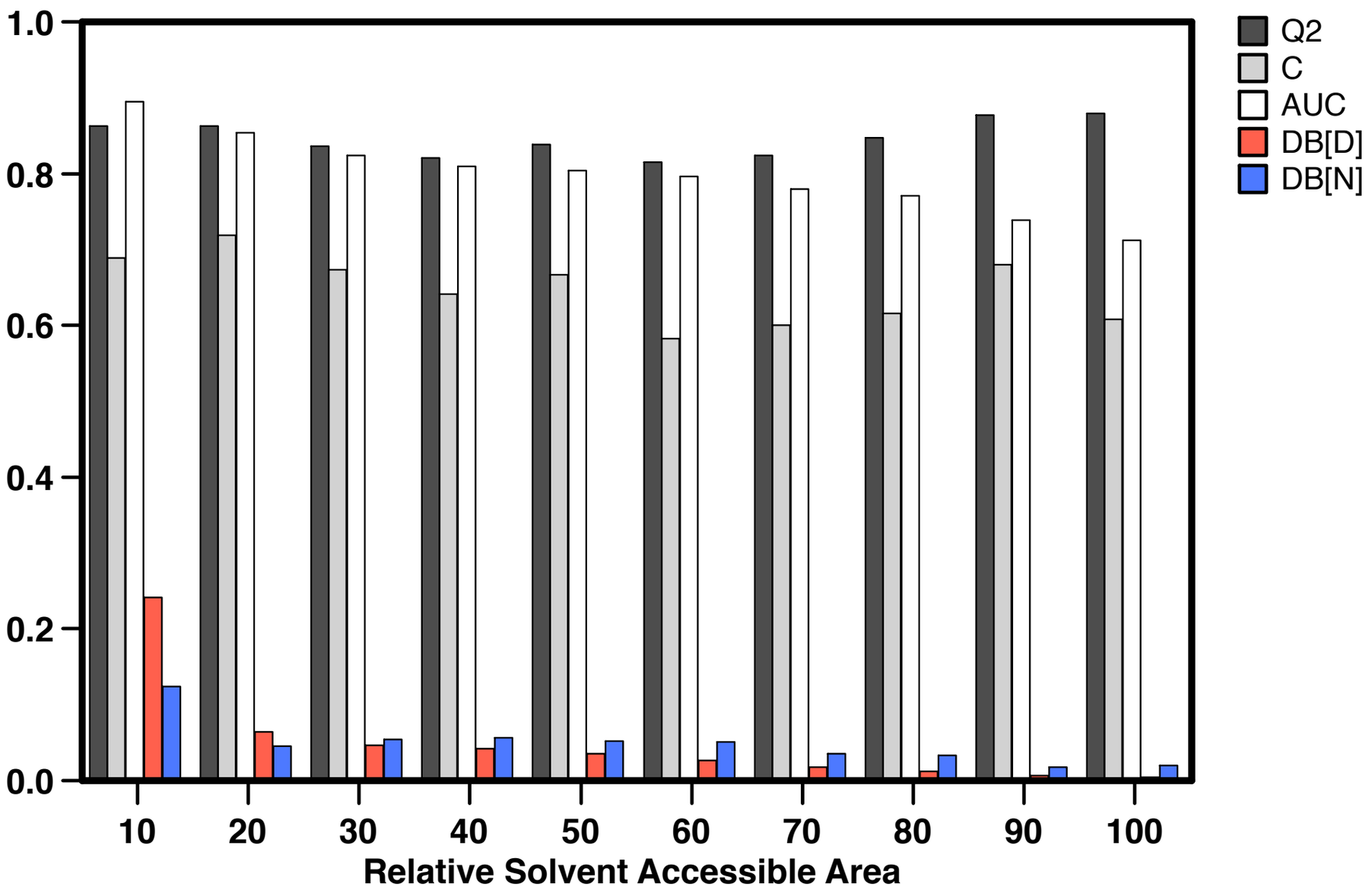
	Q2	P[D]	S[D]	P[N]	S[N]	C	AUC
SNPs&GO	0.82	0.81	0.83	0.82	0.81	0.64	0.89
SNPs&GO^{3d}	0.85	0.84	0.87	0.86	0.83	0.70	0.92





Accuracy vs Accessibility

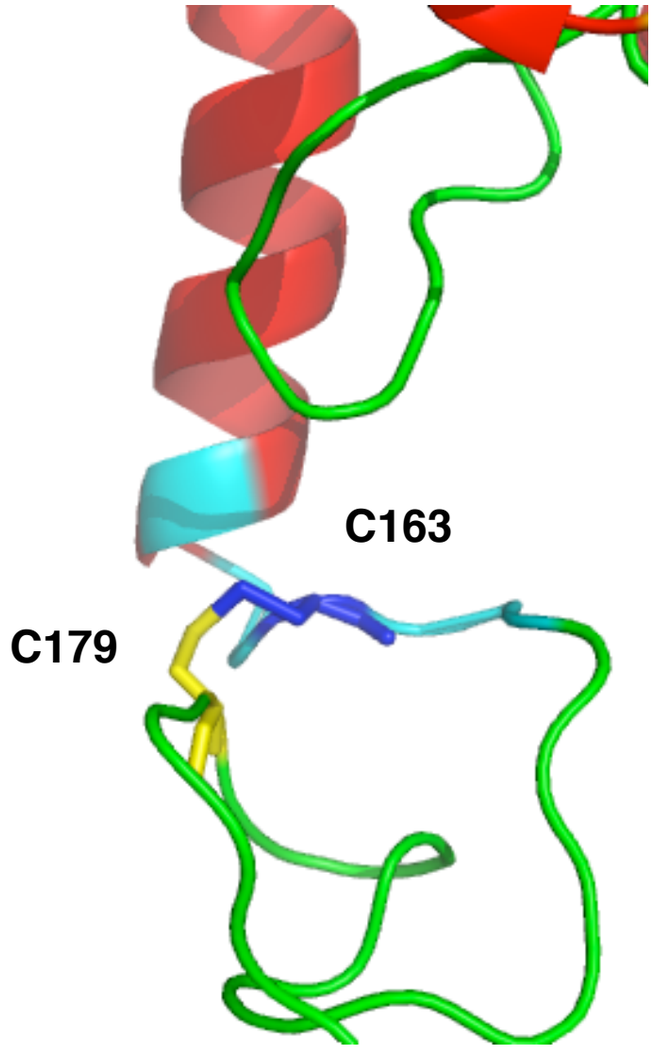
The predictions are more accurate for mutations occurring in buried region (0-30%). Mutations of exposed residues results in lower accuracy.



Prediction example

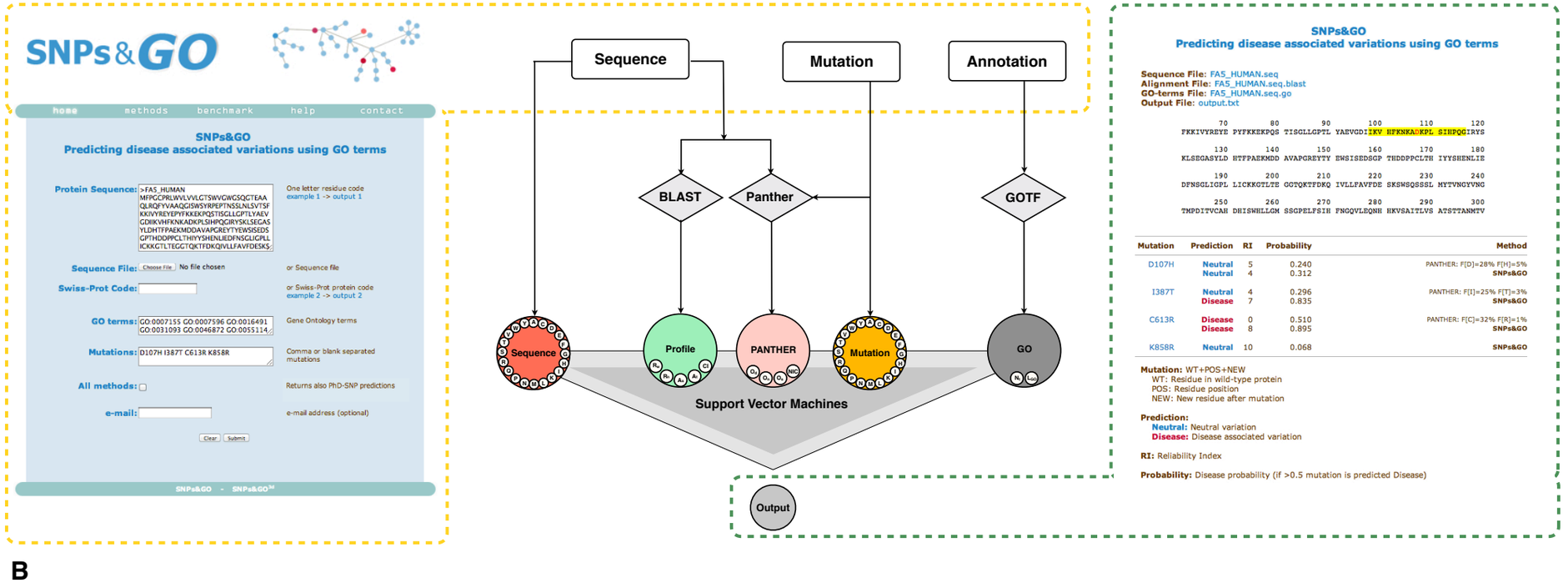
Damaging missing Cys-Cys interaction in the Glycosylasparaginase. The mutation p.Cys163Ser results in the loss of the disulfide bridge between Cys163 and Cys179. This SAP is responsible for Aspartylglucosaminuria.

1APY: Chain A, Res: 2.0 Å

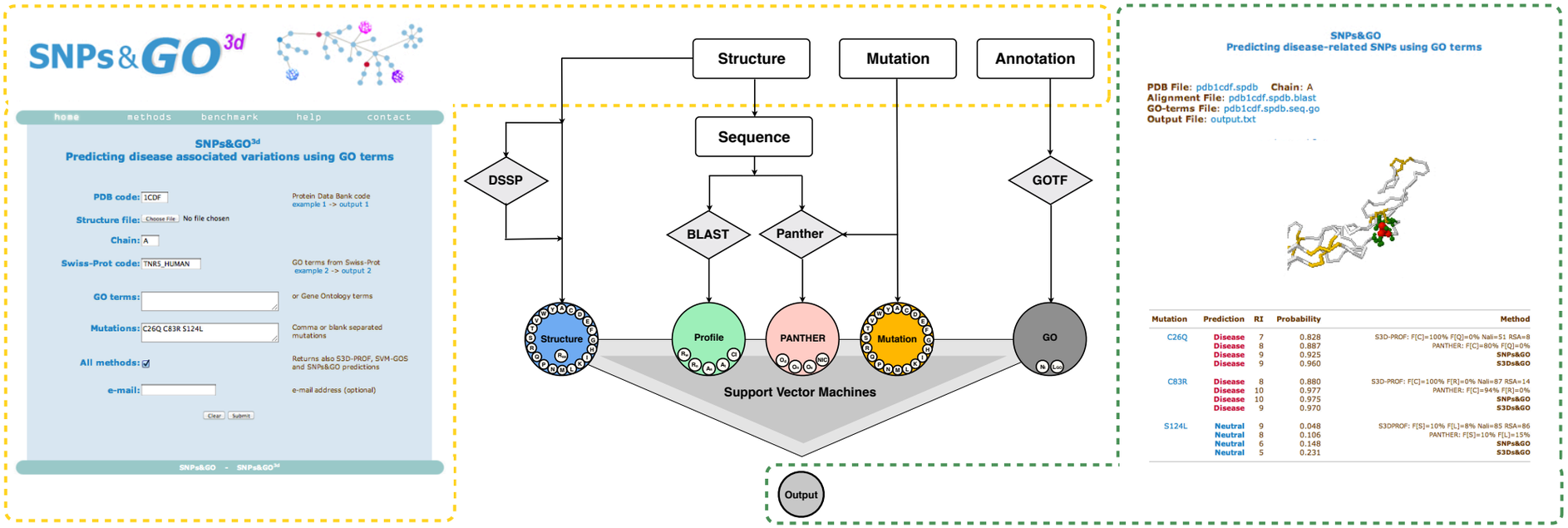


SNPs&GO web server

A



B



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

Capriotti et al. (2013). BMC Genomics. 14 (S3), S6.

SAVs Predictors

Many predictor of the effect of SAVs are available. They mainly use information from multiple sequence alignment to predict the effect of a given mutation. In his 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 disease-related and neutral polymorphisms. 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: Neutral 17883 and Disease 17883

SAVs Predictors

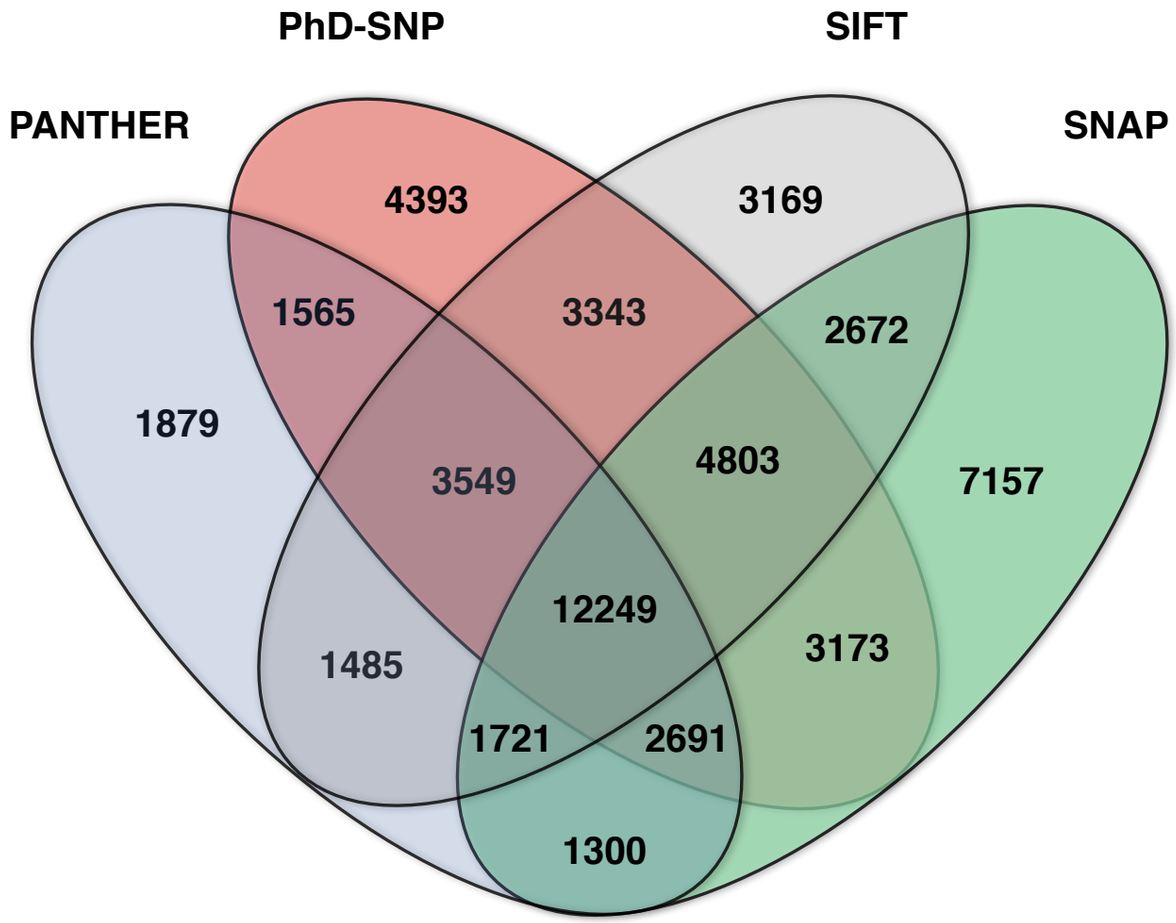
The higher correlation coefficient is between PANTHER and SIFT predictions. SNAP shows low correlation with PhD-SNP and PANTHER but higher correlation with SIFT which input is included in SNAP

C \ O	PhD-SNP	PANTHER	SNAP	SIFT
PhD-SNP	-	0.76	0.64	0.78
PANTHER	0.51	-	0.67	0.79
SNAP	0.37	0.40	-	0.69
SIFT	0.55	0.58	0.48	-

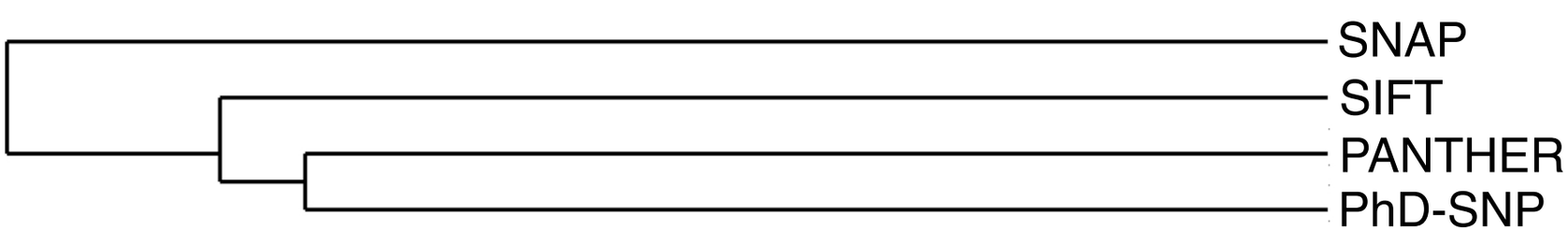
DB: Neutral 17993 and Disease 17993

Predictors tree

Using the prediction similarity we can build the predictors tree



UPGMA tree based on correlations



Prediction Analysis

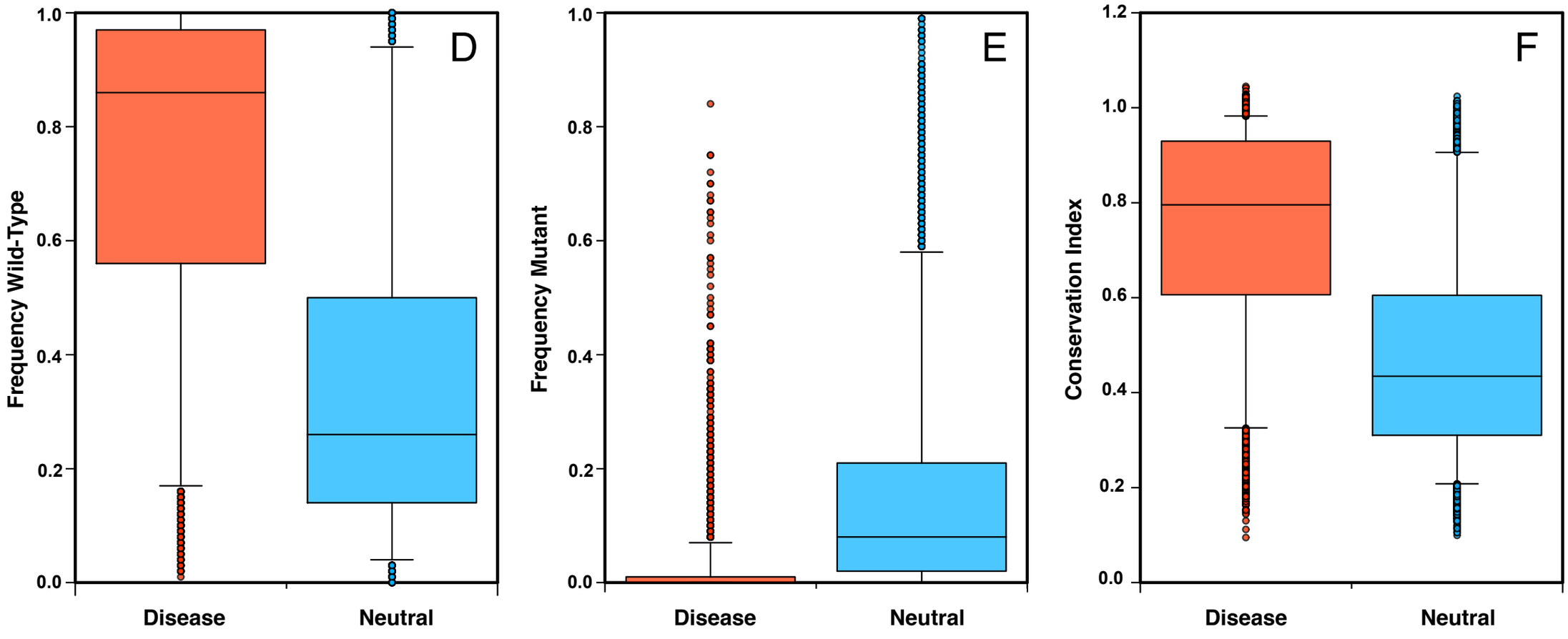
The accuracy of the predictions has been evaluated considering three different subset

- Consensus: all the predictions returned by the methods are in agreement.
- Tie: equal number of methods predicting disease and polymorphism
- 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

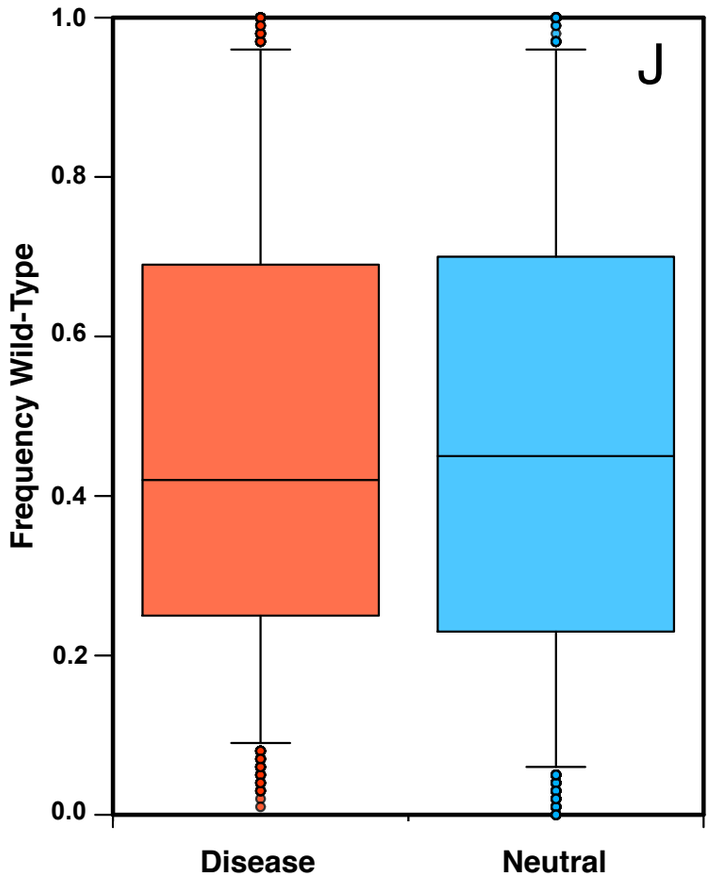
Consensus subset

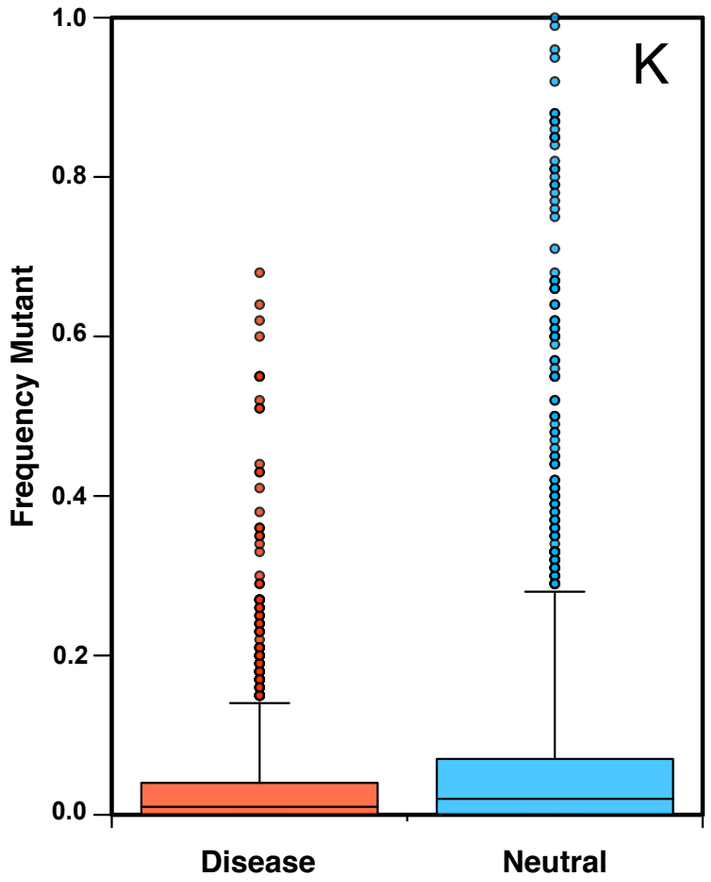
The distributions of the wild-type and new residues frequencies and CI for disease-related variants and polymorphisms on the *Consensus* subset have very little overlap.

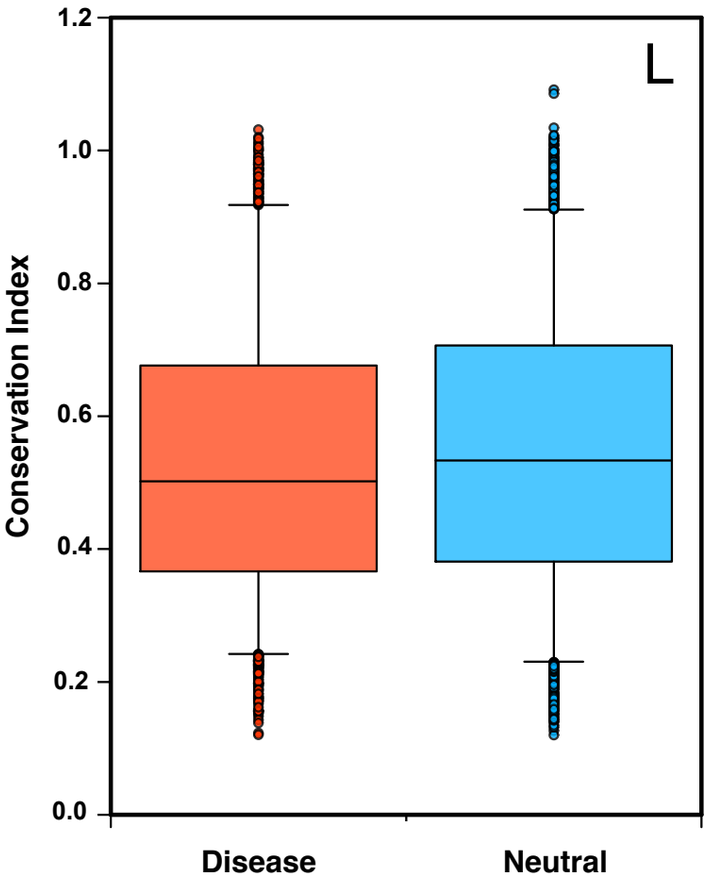


Tie subset

The distributions of the wild-type and new residues frequencies and CI for disease-related variants and polymorphisms on the *Tie* subset have almost complete overlap.

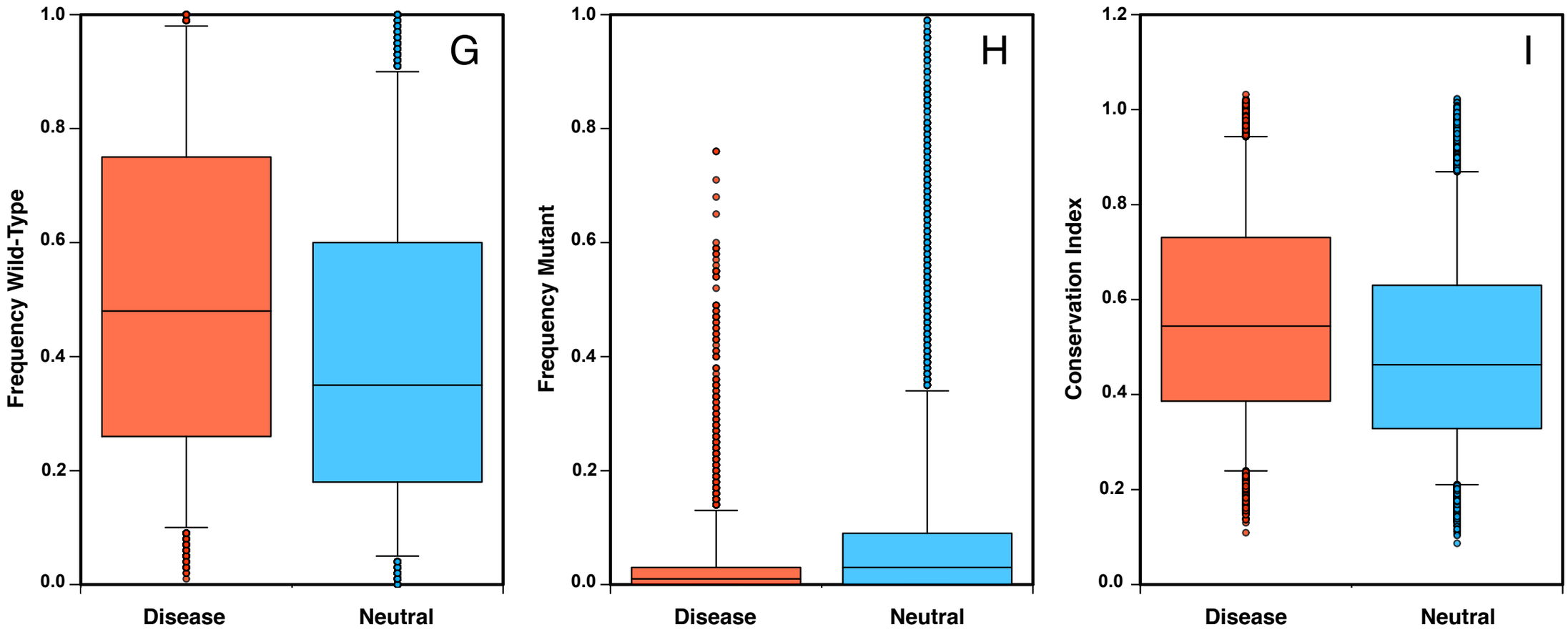






Majority subset

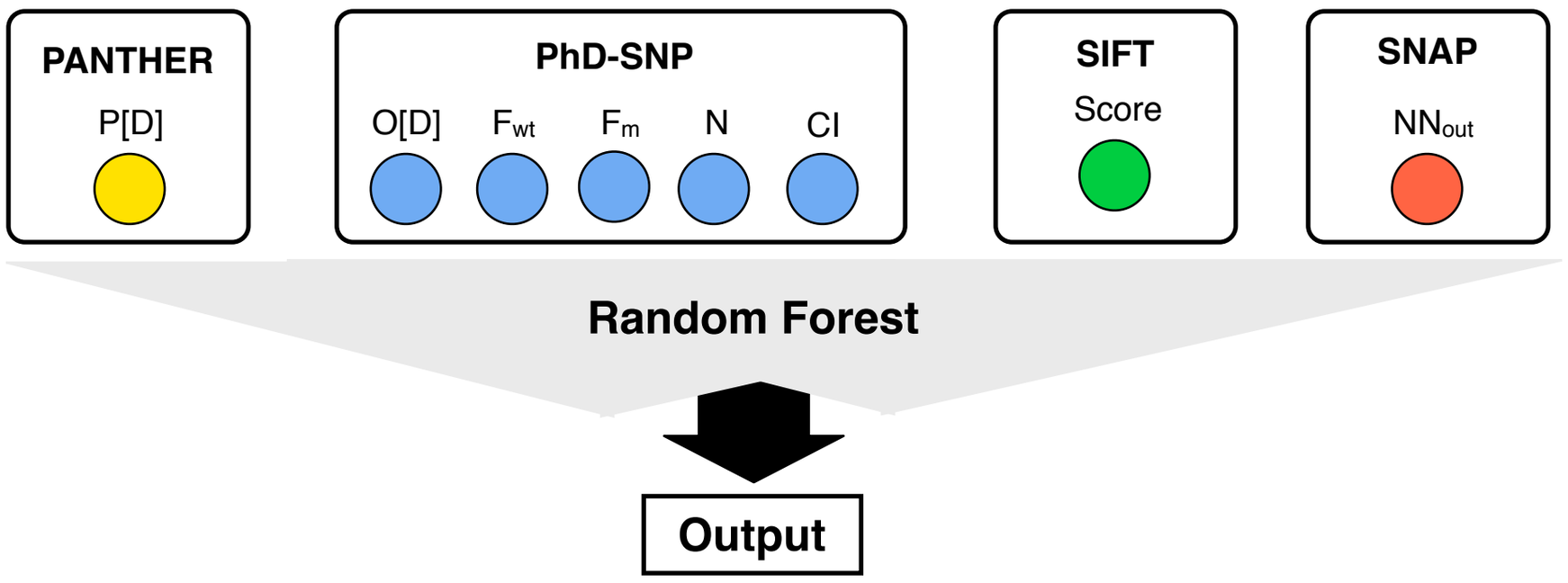
The distributions of the wild-type and new residues frequencies and CI for disease-related and polymorphism on the *Majority* subset are in an intermediate situation with respect to the previous cases.



Meta-SNP

The Meta-SNP is a RF-based meta predictor that takes in input * input features from the output of PhD-SNP, PANTHER, SNAP and SIFT.

The output of the methods can be analyzed dividing the dataset in consensus predictions (all the methods in agree), tie predictions (same number of disease and non-disease predictions) and other predictions (the remaining cases) .



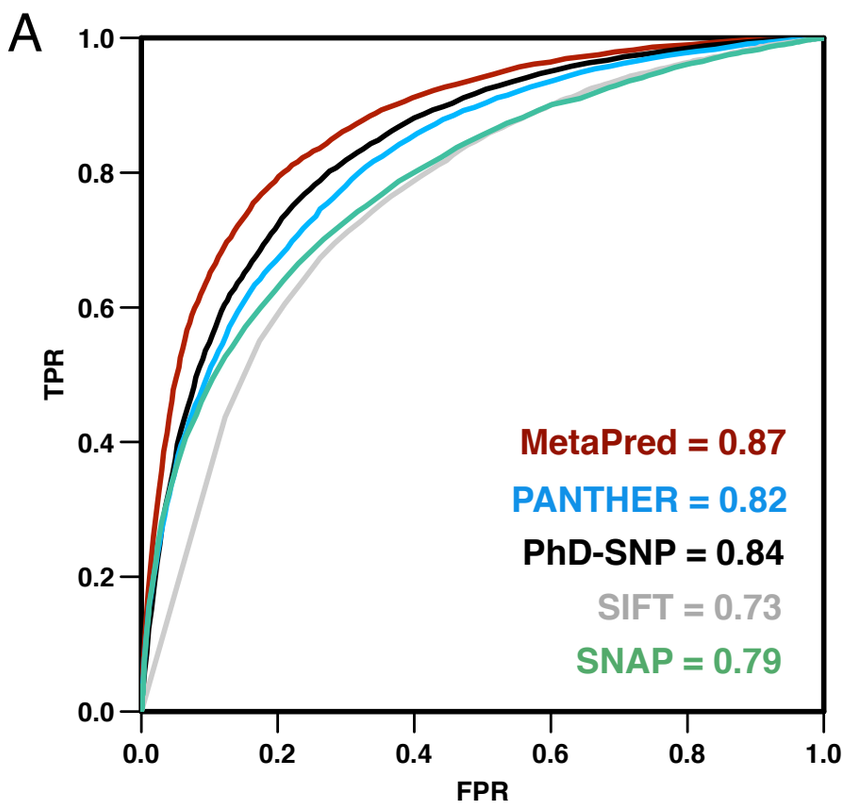
http://snps.biofold.org/meta-snp

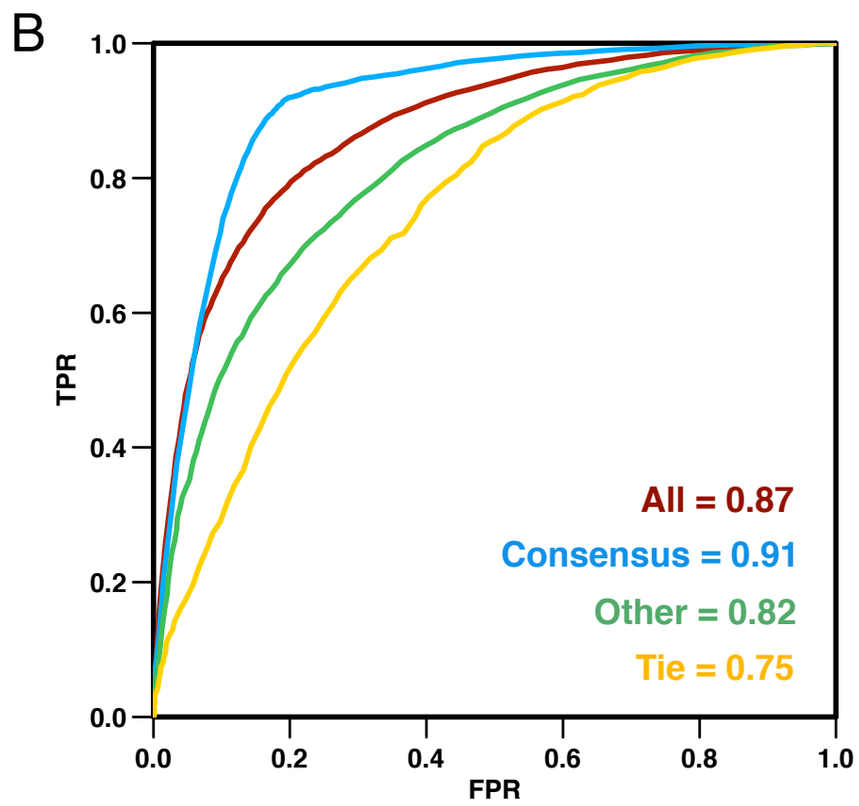
Meta-SNP accuracy

The Meta-Pred method results in better accuracy with respect to the PhD-SNP.

	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
Meta-SNP	0.79	0.80	0.79	0.79	0.80	0.59	0.87	100
Consensus	0.87	0.88	0.92	0.87	0.80	0.73	0.91	46
Majority	0.75	0.72	0.64	0.76	0.82	0.47	0.82	40
Tie	0.69	0.62	0.57	0.73	0.76	0.34	0.75	14

DB: Neutral 17993 and Disease 17993



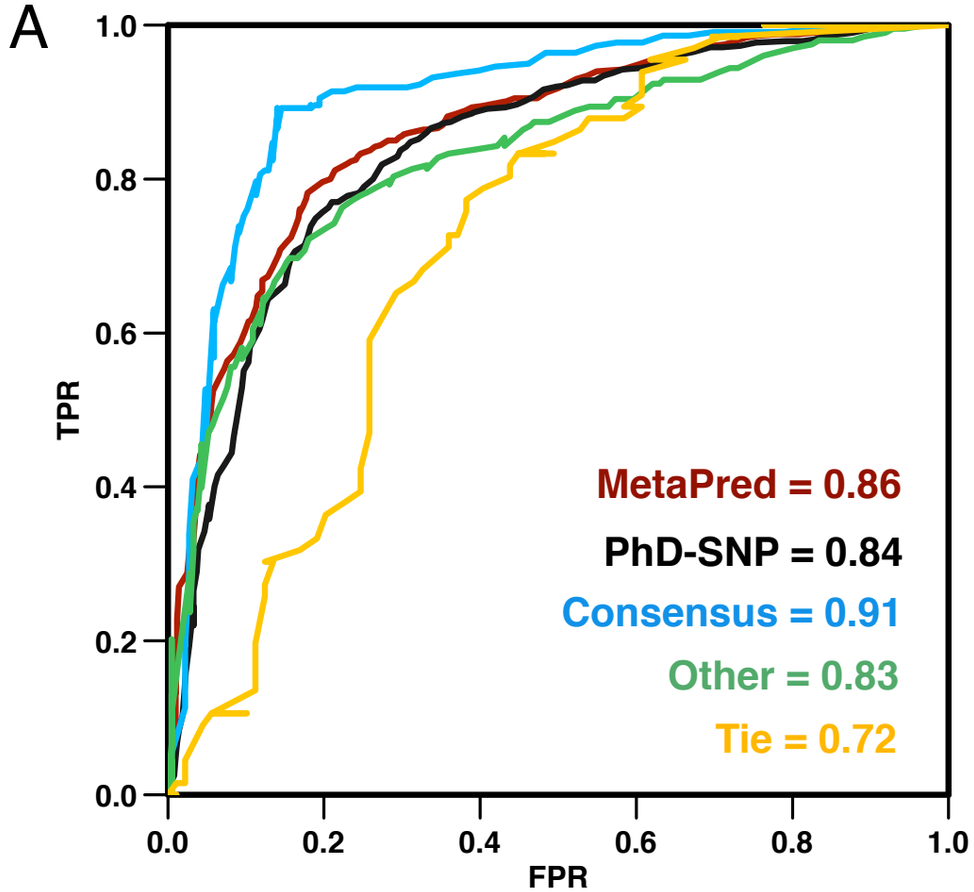


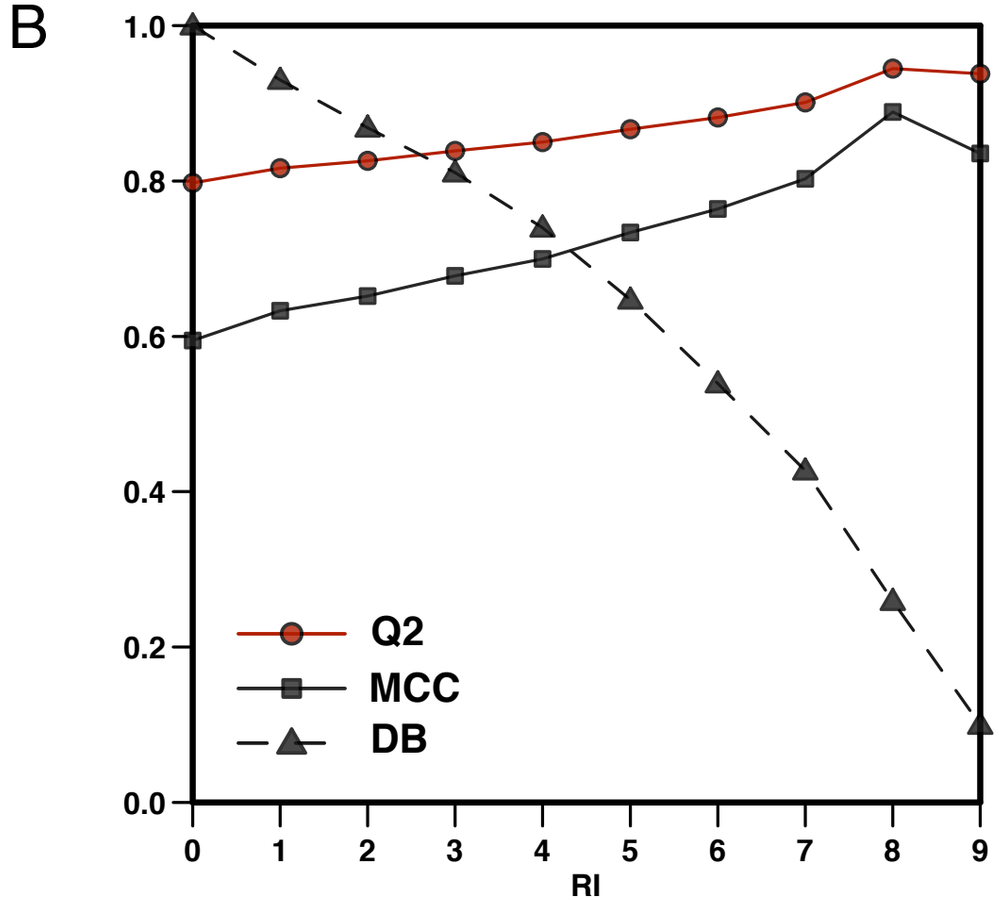
Testing Meta-SNP

Performances of Meta-Pred on the test set of 972 variants from 577 proteins

	Q2	P[D]	S[D]	P[N]	S[N]	C
Meta-SNP	0.79	0.79	0.80	0.80	0.79	0.59
PhD-SNP	0.77	0.78	0.77	0.77	0.78	0.55

DB: Neutral 486 and Disease 486

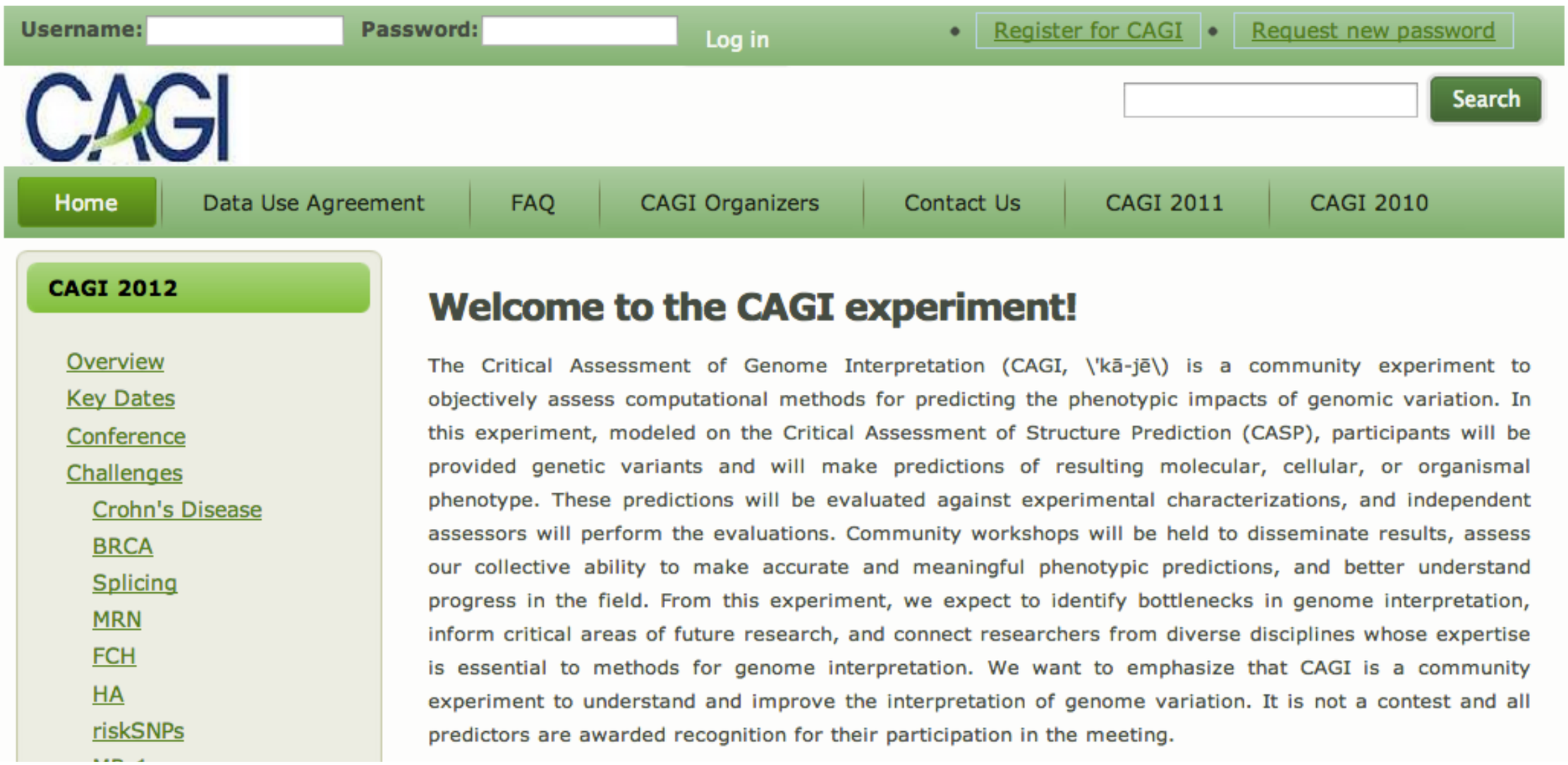




Capriotti et al. (2013). BMC Genomics. 14 (S3), In press.

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.



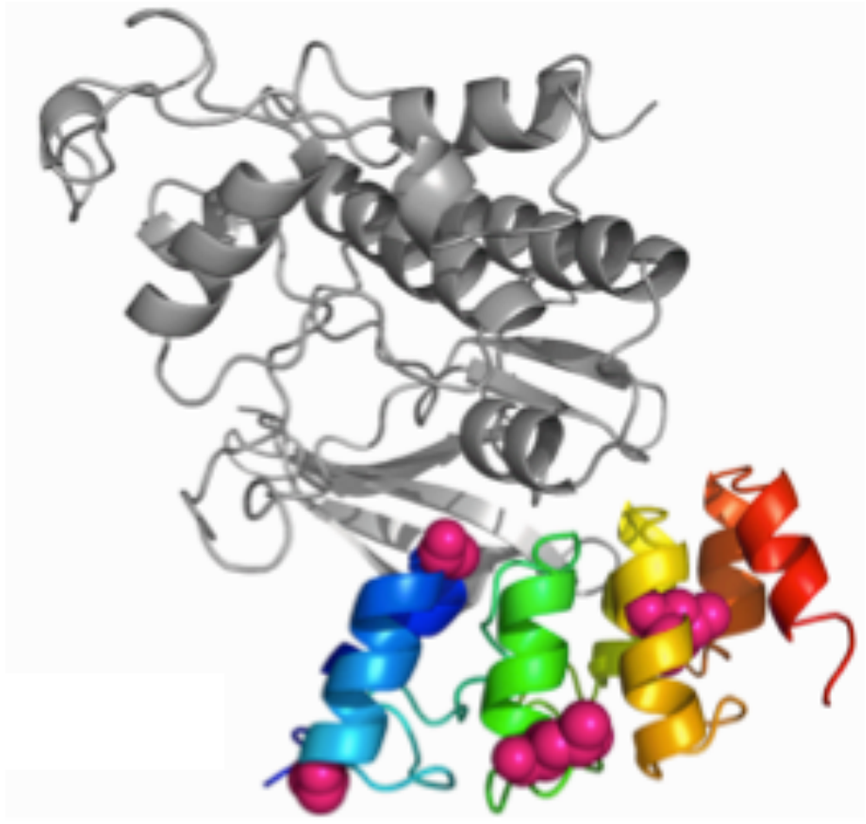
https://genomeinterpretation.org/

The CAGI P16^{INK} challenge

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

Challenge: Predict how protein variants in p16 protein impact its ability to block cell proliferation.

SNPs&GO among the best methods to blindly **predict the change in cell proliferation** associated to mutations on P16^{INK} (~70% accurate predictions).



SNPs&GO prediction

Proliferation rates have been predicted using the raw output of SNPs&GO without any fitting

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

Variants and disease

Several methods for **predicting the effect of missense single nucleotide variants**

Methods for predicting the effect of nonsynonymous single nucleotide are able to classify them in disease-causing or neutral.

The methods rely on sequence information, structure information, functional annotation. The most famous are SIFT and Polyphen but there are several other ones like PhD-SNP, SNPs&GO, Meta-Pred, MutPred, SNAP and many others

Limitations:

- the algorithms predict disease or neutral but there is no details about the disease
- available methods are not able to predict the effect of multiple genetic variants.

Datasets composition

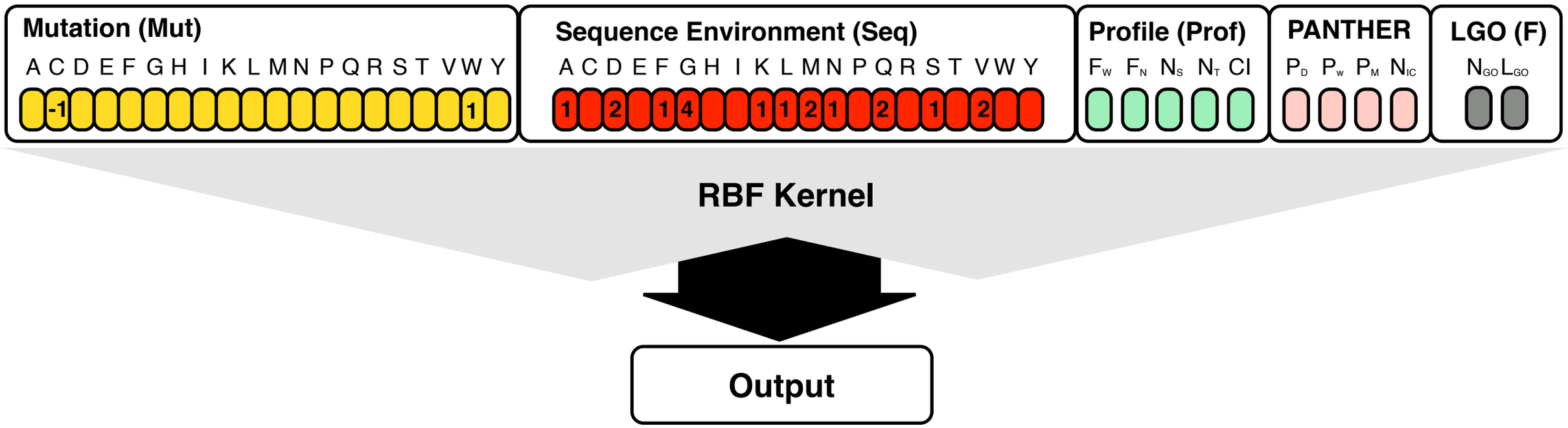
Cancer-causing mutations in CNO dataset are balanced with equal number of randomly selected neutral polymorphism In CND 50% of neutral polymorphisms are replaced with mutations related to other diseases. The Synthetic dataset contains neutral variants generated by CHASM.

Neutral / Disease	Neutral polymorphism from SwissVar	Neutral and other disease-related mutations	Synthetic Neutral
Cancer-causing Manually curated driver mutations	3163 Cancer 3163 Random Neutral (CNO)	3163 Cancer 1582 Other Diseases 1581 Random Neutral (CND)	3163 Cancer 3163 Synthetic (Synthetic)

Cancer-specific method

A SVM based method (SPF-Cancer) similar to a previous developed one has been proposed to predict if a given SNP is related/associated to cancer.

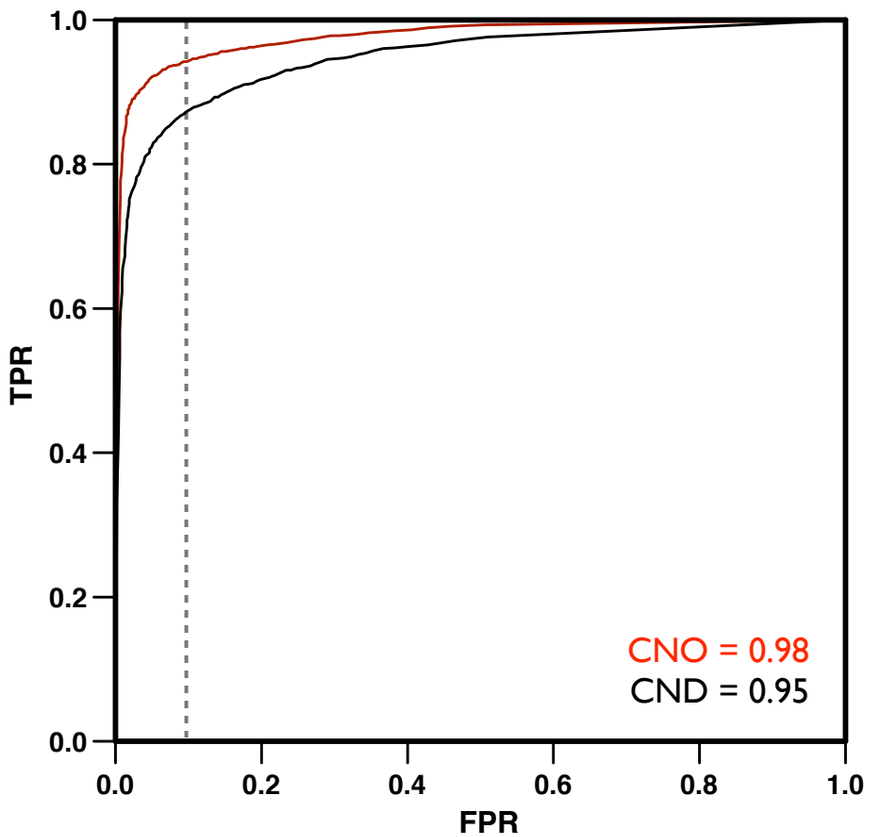
The method takes in to account 4 different types of information encoded in a 51 elements vector. The input features are: mutation data; sequence environment, sequence profile, PANTHER output and functional score based on GO terms.

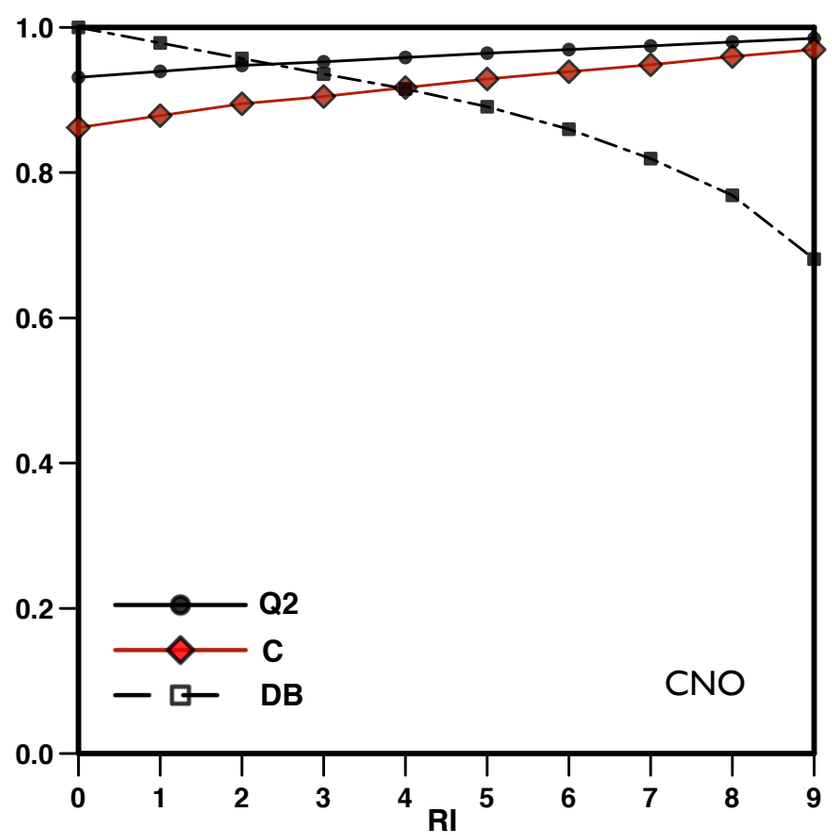


Method accuracy

Two tests: Cancer + Neutral (CNO) and Cancer + Neutral and other Diseases (CND)

	Q2	P[D]	S[D]	P[N]	S[N]	C	AUC
CNO	0.93	0.93	0.93	0.93	0.93	0.86	0.98
CND	0.90	0.87	0.93	0.92	0.86	0.79	0.95

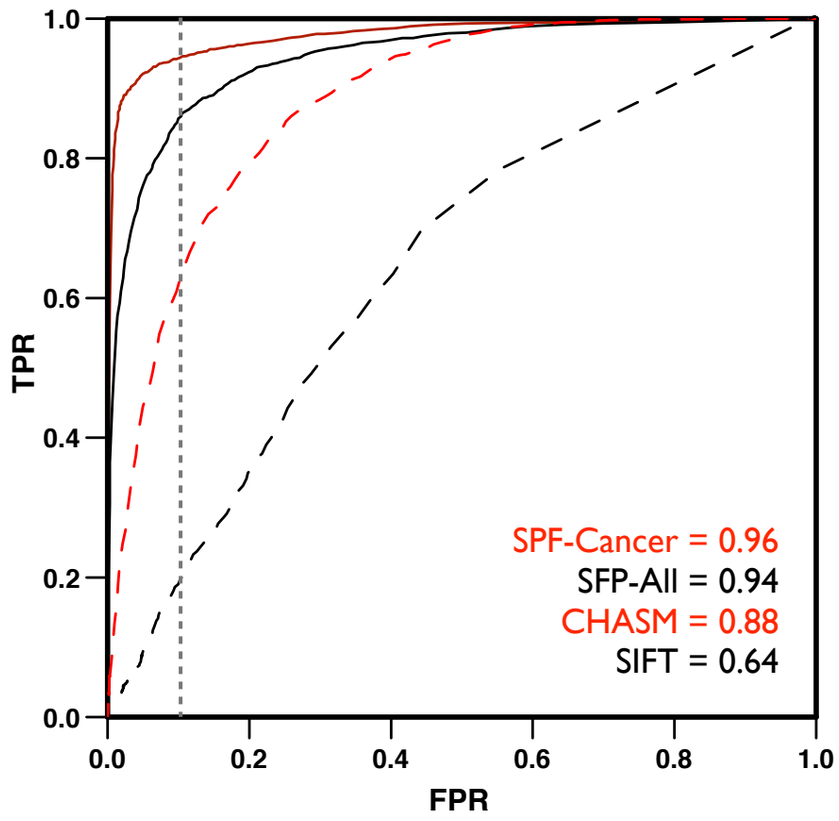




Q2=(TP+TN)/N **P[i]**=TP[i]/(TP[i]+FP[i]) **S[i]**=TP[i]/(TP[i]+FN[i])
AUC=Area under ROC curve **C**=Matthews Correlation Coefficient

Benchmarking

SPF-Cancer results in higher accuracy and correlation than the other available methods covering the 100% of the dataset (see column PM). SPF-Cancer is more accurate than similar method with GO score calculated using the whole mutation set. This is more evident when the results of the two GO-score based methods are compared on the set containing other disease related mutations (CND)

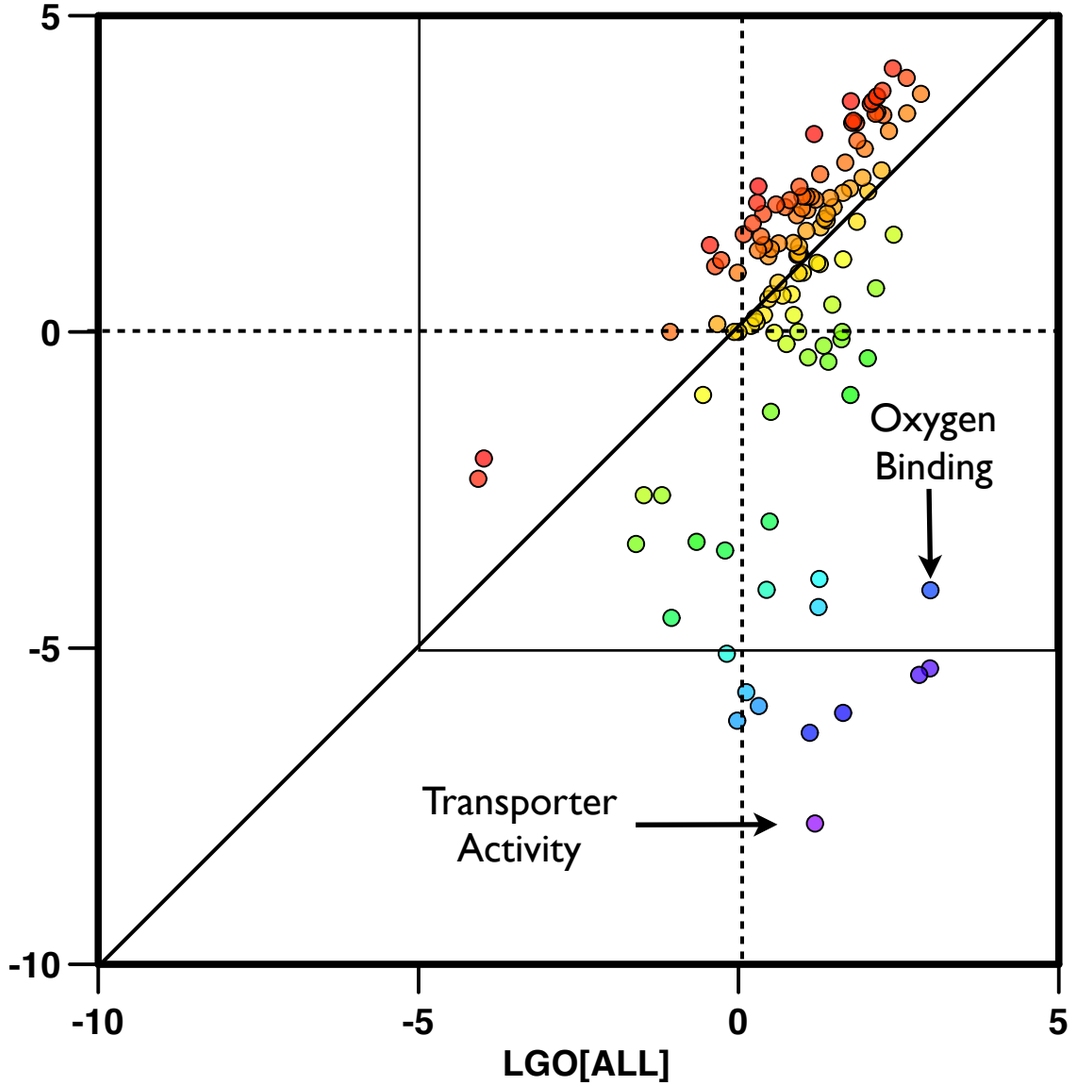


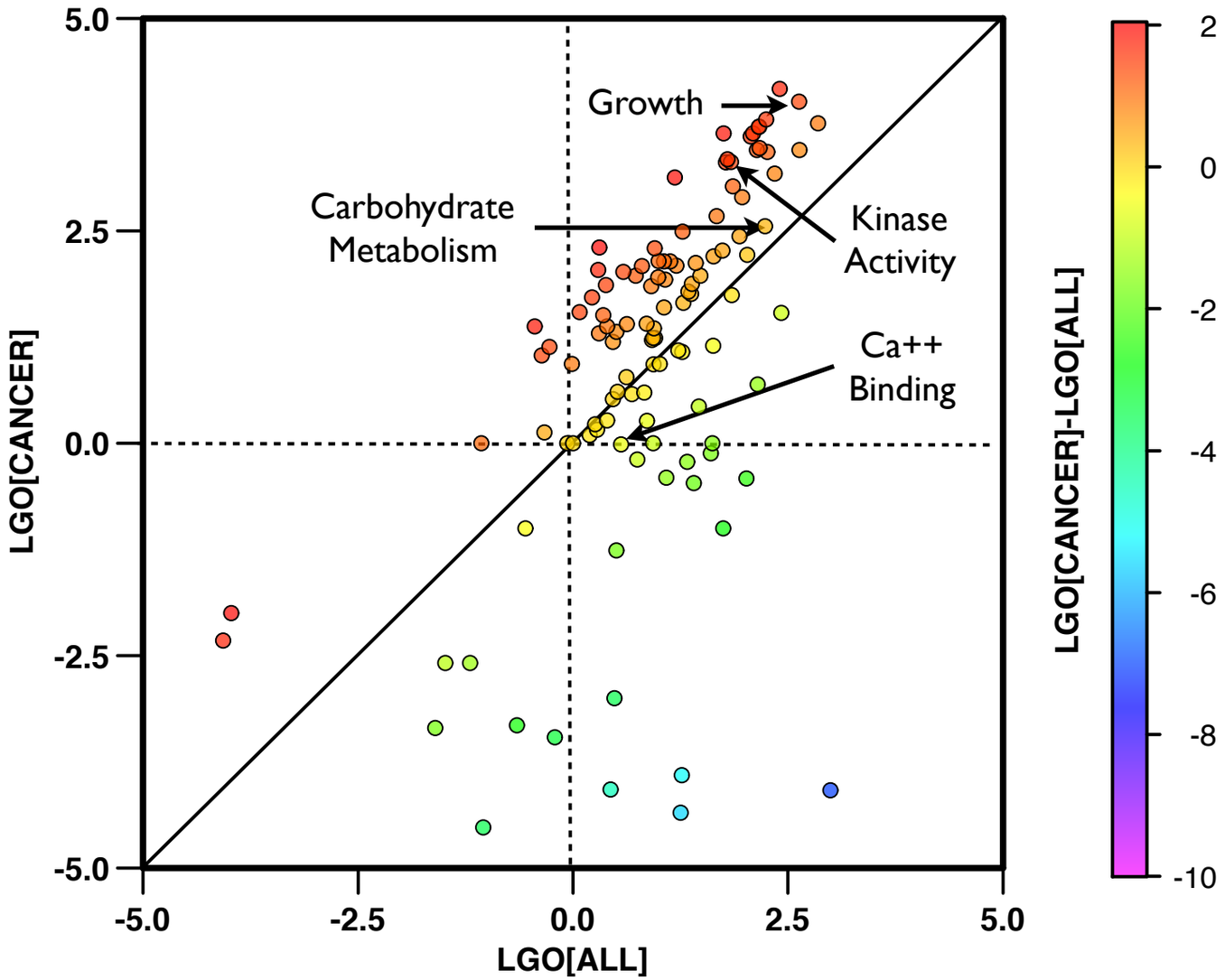
	Q2	P[D]	S[D]	P[N]	S[N]	C	AUC	PM
SIFT	0.61	0.62	0.66	0.60	0.56	0.22	0.64	95
CHASM	0.80	0.85	0.73	0.76	0.87	0.60	0.88	100
SPF-All	0.88	0.88	0.87	0.87	0.88	0.75	0.94	100
SPF-Cancer	0.90	0.91	0.90	0.90	0.91	0.81	0.96	100

Synthetic: 3163 cancer-causing and 3163 synthetic neutral variants

GOslim scores

The subset of cancer causing proteins is enriched for Growth and Kinese Activity. Functions related to Oxygen Binding and Transporter Activty are less represented.

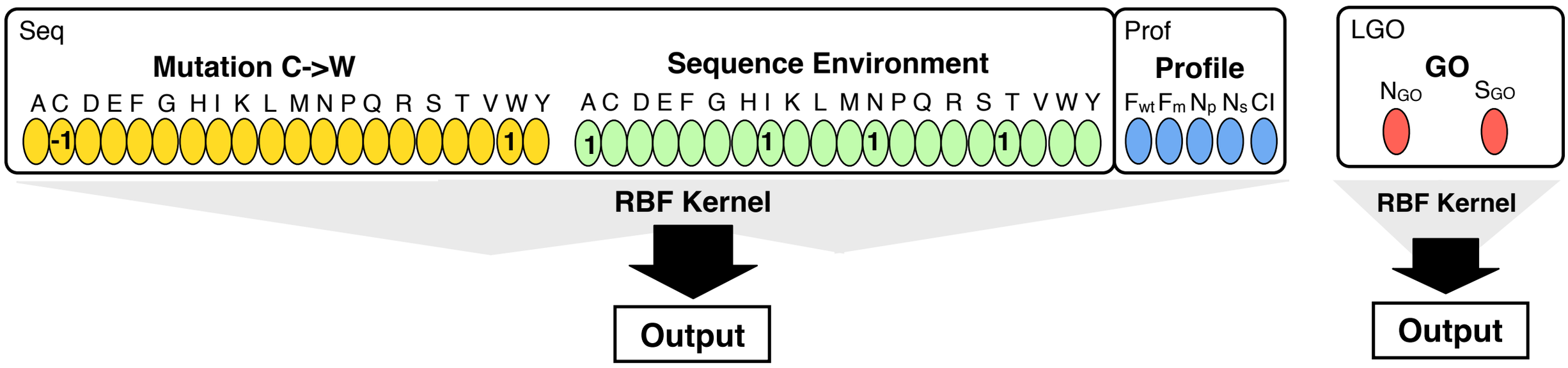




Capriotti E, Altman RB. (2011). Genomics. 98; 310-317

Prediction evaluation

To evaluate the results of our prediction, we divided the input features in two parts: the mutation input features (Seq+Prof) including the mutated residue, the sequence environment and the sequence profile in the mutated position and the sequence dependent functional information (GO).



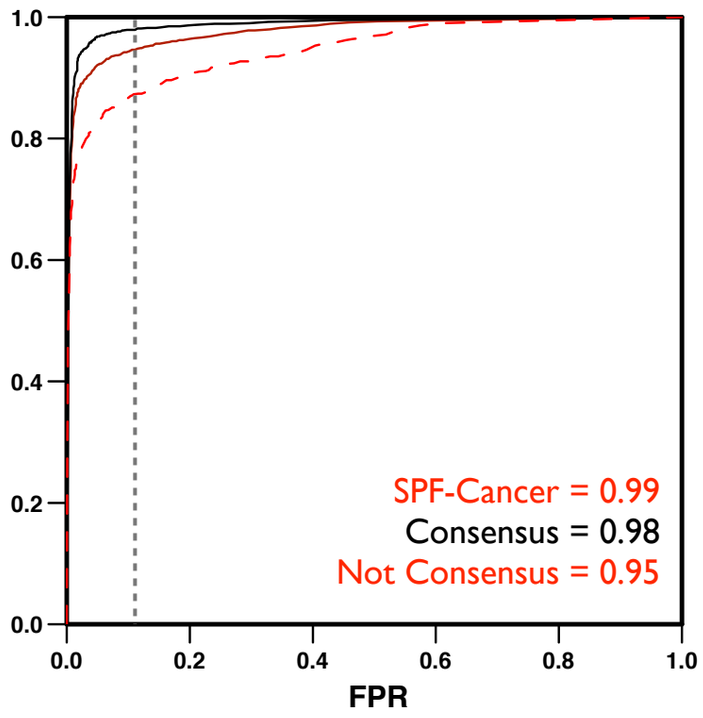
Two independent predictors have been 22 and predictions are divided according to the fact that both methods (Seq+Prof, GO) predict or not. For agreeing and not agreeing predictions we tested the performance of our method.

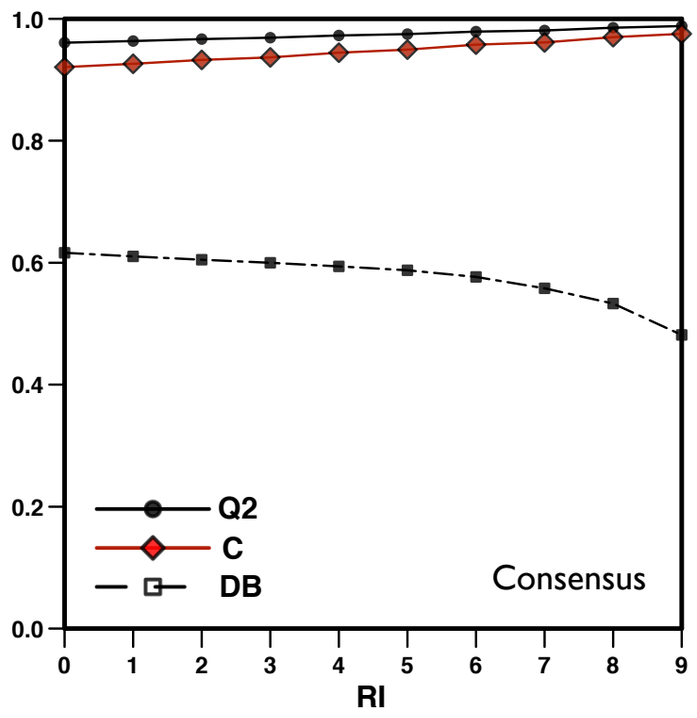
http://snps.biofold.org/drcancer

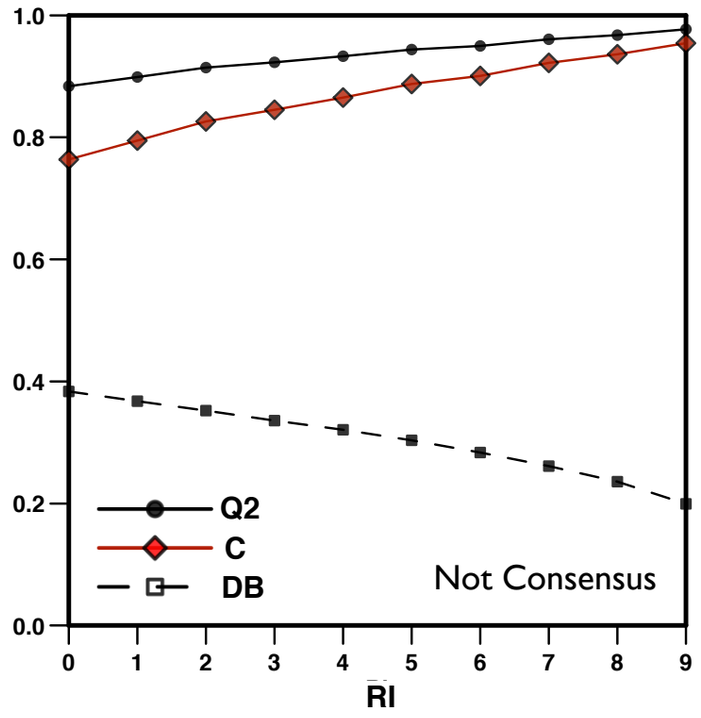
Prediction accuracy

Consensus predictions are more accurate than not agreeing ones. If 10% of true positive are accepted agreeing predictions (~62%) results in 94% of true positive. This is a good value if compared with 87% true positive for not Consensus predictions (~38%).

	Q2	P[D]	S[D]	P[N]	S[N]	C	AUC	PM
SPF-Cancer	0.93	0.93	0.93	0.93	0.93	0.86	0.98	100
Consensus	0.96	0.96	0.95	0.96	0.97	0.92	0.99	62
notConsensus	0.88	0.90	0.90	0.87	0.87	0.76	0.95	38







Q2=(TP+TN)/N **P[i]**=TP[i]/(TP[i]+FP[i]) **S[i]**=TP[i]/(TP[i]+FN[i])
AUC=Area under ROC curve **C**=Matthews Correlation Coefficient

Conclusions

- Evolutionary information are important for the prediction of deleterious variants. The wild-type residues in disease-related mutation sites are more conserved than in neutral sites.

- Different algorithms for genome interpretation predicting the effect of a single amino acid polymorphism on human health have been tested. The methods based on functional information are among the most accurate.

- Relative solvent accessible area and structure environment are better features than sequence-based ones to discriminate between disease-causing and neutral mutants.

- Disease-specific GO term score can be used to detect deleterious variants related to the specific disease class. In the case of cancer-causing mutations this strategy allowed to improve the quality of the predictions.

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