GPSR

A resource for genomics, proteomics and systems biology

Integration of Computer Programs Developed at

GPS Raghava's Group

Bioinformatics Centre,
Institute of Microbial Technology, Chandigarh

Message to Users.....

The field of computational biology has witnessed tremendous change in the years gone by. In its initial infancy stage, computation biology was used to solve only smaller biological problems. However, with the advancement in the field, scientists started using computational biological techniques heavily for solving even complex problems like protein modeling. In present era, computational biology is dominated by bioinformatics where managing, analyzing and mining biological data is a major challenge. And one of the major challenges for any computer or bioinformatics professional is to understand need of biologist and develop user-friendly software.

When I joined Institute of Microbial Technology (IMTECH), Chandigarh in 1986 as computer scientist, my primary duty was to provide computer services to IMTECH. At that time computer programs were developed for automation of institute. In 1990, first scientific program ELISA_eq was developed for computing antigen/antibody concentration from ELISA data in GW-BASIC. From 1990-93 computer programs for well defined biological problems like mapping restriction sites, calculation of DNA/protein size were also developed. During 1993-98, majority of computer programs were developed either for predicting proteins tertiary structure or for benchmarking the alignment methods. All these programs were standalone programs, developed for DOS/Windows using programming various languages like FORTRAN, PASCAL, C. These programs were distributed free for academic users via floppy or CD. Though these programs were user-friendly, but one needs to have a hardware/software compatibility and knowledge of installation, in order to run them. To overcome this problem, we started developing web services instead of standalone softwares. To use these webservers one only needs to have a computer with browser and access to internet.

In 1998, I established my group with few PhD students having an objective to solve biological problems. In last ten years our group has developed more than 100 web servers in the field of bioinformatics. These web servers can be grouped in following main categories

- Subunit vaccined design of epitope basec vaccine;
- Genome annotation (prediction of gene, repeat, polyadylation sites etc.).
- Functional annotation of proteomes.
- Computer-aided drug discovery.

These web servers are heavily used (≥ 20,000 hits per day) and cited (≥ 1500 citation) by scientific community worldwide.

One of the major challenges for bioinformaticians is to understand and solve biologist's problems. At the same time the solution should be such that it is user-friendly so that a person having just a little knowledge of computer can also utilizes it. Though we have tried our best to help the biologist, our programs/services are still far from perfect. Our webservers perform well for single sequence queries or for a small number of sequences but they are unable to perform predictions for the whole genome or proteome (because we can't provide the required CPU time). Moreover, many a times due to the limitation of available bandwidth and other security reasons, users wish to run these servers on their local machines. In an urge to comply with these demands our group is releasing this software package, which is a collection and integration of computer programs developed at our group over the years.

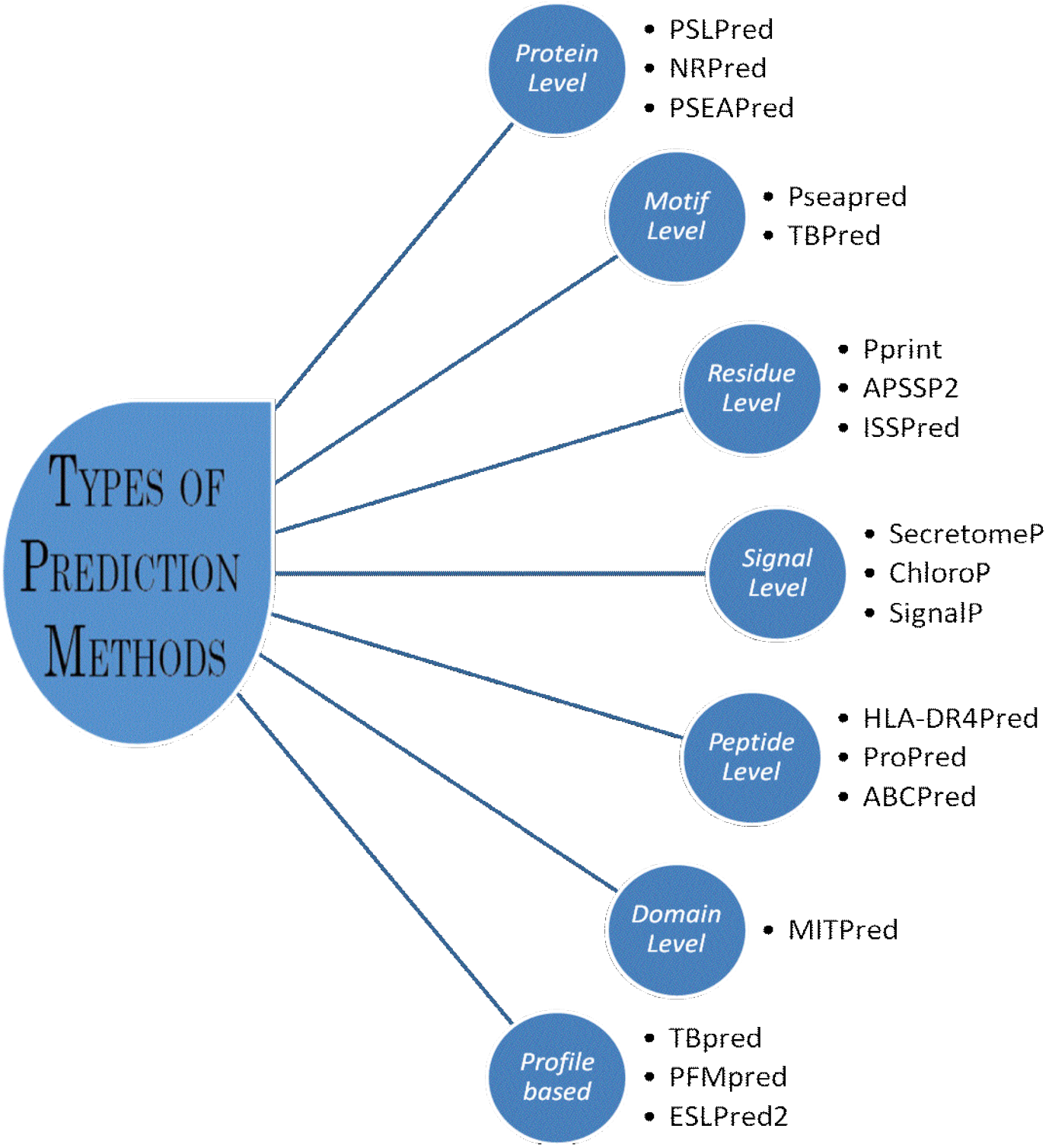
The manual of this package has three major sections; first section is written for students working in the field bioinformatics particularly for software developers. This section describes I) commonly used major computational tools, frequently used for developing bioinformatics tools; ii) type of prediction methods and iii) procedure for evaluating of a newly developed method. Second section is written for users who wish to analyze the proteins. In this section, all small programs is described which are commonly used for building major software packages. Third section describes stanalone programs based on our servers/methods, important for users who want to run our methods on whole proteome. I wish all the best for our users.

(G.P.S. Raghava)

Disclaimer and Copyright

The programs and the package are free softwares for academic users. Permission to use, copy, and modify any part of this software for educational, research and non-profit purposes is hereby granted but distribution to third-party is prohibited. They are distributed in the hope that they will be useful but WITHOUT ANY WARRANTY; without even the implied warranty of MERCHANTABILITY or FITNESS FOR A PARTICULAR PURPOSE. If you want to include this software in a commercial product, please contact at raghava@imtech.res.in.

Types of Prediction Methods


Types of Prediction Methods
Protein Level
PSLPred
NRPred
PSEAPred
Motif Level
Pseapred
TBPred
Residue Level
Pprint
APSSP2
ISSPred
Signal Level
SecretomeP
ChloroP
SignalP
Peptide Level
HLA-DR4Pred
ProPred
ABCPred
Domain Level
MITPred
Profile based
TBpred
PFMpred
ESLPred2

1. Prediction at Protein Level:

These methods are developed to predict overall function of charactestics of proteins. In these methods we used complet protein as input. Following are few examples

1.1 Subcellular level prediction:

The cellular localization of a protein is one of the most fundamental properties of any protein due to cellular division of labour. The correct prediction of subcellular location can be a major breakthrough for functional prediction, since to perform a function, protein must be located in their native location, such as nucleus or mitochondria or outside the cell in case of secretory proteins. The native subcellular localization of a protein is one of the indicators of protein function.

Over the years numbers of methods have been developed for the prediction of subcellular localization in prokaryotes as well as eukaryotes. Existing subcellular localization methods can be divided into various categories:

1. **Similarity search based techniques:** query sequence is searched against experimentally annotated proteins.
   Limitation: fail to predict new/novel proteins, if query protein does not have similarity with known proteins.
2. **Signal sequence based techniques:** number of methods fall under this category in which leader sequence or sorting sequence present on protein itself is used for prediction. E.g. TargetP, PSORTb, SignalP
3. **Sequence composition based techniques:** number of methods has been developed so far based on the sequence composition. *e.g.* SubLoc, NNPSL
4. **Organism specific and location specific subcellular localization prediction:** Organism specific approach is more useful than generalised approach.

Methods

Several computational tools for predicting the subcellular localization of a protein are publicly available, a few of which are listed below:

Methods	**Techniques Used**
PSLpred	Composition based+SVM+PSI-BLAST
NRpred	Composition based
GPCRpred	Composition based
ESLpred	SVM+Dipeptide Composition+PSI-BLAST

SRTpred	Composition based+physic-chemical properties+PSI-BLAST
Cytopred	SVM+PSI-BLAST hybrid approach
PSEApred	SVM
PFMpred	SVM
HSLpred	Composition based+SVM+PSI-BLAST
NNPSL	Neural Network

Relevance

Determining subcellular localization is important for understanding protein function and is a critical step in genome annotation. Knowledge of the subcellular localization of a protein can significantly improve target identification during the drug discovery process. For example, secreted proteins and plasma membrane proteins are easily accessible by drug molecules due to their localization in the extracellular space or on the cell surface.

Subcellular localization prediction allows researchers to make inferences regarding a protein's function, to annotate genomes, to design proteomics experiments and—particularly in the case of bacterial pathogen proteins—to identify potential diagnostic, drug and vaccine targets.

1.2 Class level prediction:

1.2.1 Classification of proteins

GPCRsclass: classification of amine type of G-protein-coupled receptors

1.2.2 Nucleotide binding protein prediction:

Most of the functions of DNA/RNA are performed through interaction with proteins. Prediction of DNA/RNA binding Proteins can be categorised into 2 categories:

Structure based methods: structure based methods can't be used in high throughput annotation, as they require the structure of a protein for the prediction

Sequence based methods: Only couple of sequence based prediction methods have been developed so far. These methods are based on pseudo-amino acid composition, amino acid composition, composition of physico-chemical properties and Support Vector Machine (SVM).

METHODS:

DISIS: predicts DNA binding sites directly from amino acid sequence

DBS-Pred: predict DNA-binding proteins using amino acid composition

1.3 Family level prediction

Computational prediction and classification of GPCRs can supply significant information for the development of novel drugs in pharmaceutical industry.

GPCRpred: An SVM Based Method for Prediction of families and subfamilies of G-protein coupled receptors

GSTPred: prediction of GST proteins

GPCRsIdentifier: a corresponding stand-alone executable program for GPCR identification and classification.

1.4 Structure class of proteins

Proclass: predict the class of protein from its amino acid sequence

TBBpred: predicts the transmembrane Beta barrel regions in a given protein sequence

2. Prediction at Residue level:

2.1 Prediction of Nucleotide binding residues:

Structural and physical properties of DNA provide important constraints on the binding sites formed on surfaces of DNA-binding proteins. Characteristics of such binding sites may be used for predicting DNA-binding sites from the structural and even sequence properties of unbound proteins. This approach has been successfully implemented for predicting the protein-protein interface. Here, this approach is adopted for predicting DNA-binding sites in DNA-binding proteins. First attempt to use sequence and evolutionary features to predict DNA-binding sites in proteins were made by Ahmad et al. (2004) and Ahmad and Sarai (2005). Some methods use structural information to predict DNA-binding sites and therefore require a 3-dimensional structure of the protein, while others use only sequence information and do not require protein structure in order to make a prediction. Structure- and sequence-based prediction of DNA-binding residues in DNA-binding proteins can be performed on several web servers listed below:

1. **Pprint** (**P**rediction of **P**rotein **R**NA- **Int**eraction): is a web-server for predicting RNA-binding residues of a protein. The prediction is done by SVM model trained on PSSM profile generated by PSI-BLAST search of 'nr' protein database.

2.2 Post-translational modifications of proteins

ISSPred: Intein Splice Site Prediction

DictyOGlyc: O-(alpha)-GlcNAc glycosylation sites (trained on Dictyostelium

discoideum proteins)
NetAcet: N-terminal acetylation in eukaryotic proteins
NetCGlyc: C-mannosylation sites in mammalian proteins
NetCorona: Coronavirus 3C-like proteinase cleavage sites in proteins
NetNGlyc: N-linked glycosylation sites in human proteins
NetOGlyc: O-GalNAc (mucin type) glycosylation sites in mammalian proteins
NetPhos: Generic phosphorylation sites in eukaryotic proteins
NetPhosBac: Generic phosphorylation sites in bacterial proteins
ProP: Arginine and lysine propeptide cleavage sites in eukaryotic protein sequences

2.3 Secondary structure prediction:
APSSP2: Advanced Protein Secondary Structure Prediction Server

2.4 Turn prediction:
BhairPred: SVM based method for prediction of beta-hairpins in proteins
BTEVAL: Evaluation of beta turns prediction methods
BetaTPred: predicting ß-turns in a protein from the amino acid sequence
BetaTPred2: Prediction of ß-turns in proteins using neural networks and multiple alignments
Betaturns: Prediction of beta-turn types
AlphaPred: predicts the alpha turn residues in the given protein sequence
GammaPred: predicts the gamma turn residues in the given protein sequence

RELEVANCE:
Useful application of DNA-binding residues prediction would be the identification of proteins that bind to DNA. Recognition of probable binding sites both on the protein and the DNA will go a long way in diagnosing the basis of these interactions. Their discovery can help lead subsequent works such as site-directed mutagenesis and constrained macromolecular docking. Prediction of functional sites to act as filters in a predictive scheme for docking can be as effective as manually introducing biological constraints.

The identification of DNA-binding sites can also assist in prediction of DNA-binding behaviour of a protein. This is similar in spirit to other studies that assign functions to a protein on the basis of functional sites discovered on its surface, such as protein-protein and protein-DNA interaction sites.

3. Prediction at peptide/epitope level:
The potential importance of epitope identification in developing vaccines against

infectious, immune and other antigen-related diseases, epitopes are studied widely by researchers in various fields, and a large expansion of databases, predictive methods and software focussing on different types of epitopes has been witnessed. The average immunologists are overwhelmed with such a broad array of immunological analysis tools that are highly specific in use, not well understood or defined, tested on limited data and not publicly accessible

Epitope prediction dates back to 1981 when the first B cell epitope prediction method was developed by Hopp and Woods. Since then many more methods have been developed or adapted from other computational tools; for example B cell epitope prediction and T cell epitope prediction. Despite the early start, however, prediction systems for B cell epitopes are still in their infancy.

General epitope prediction methods

1. **Sequence-based epitope prediction**

   Sequence-based method utilises the notion that sequence dictates structure and identical structure in turn leads to identical functions. T cell epitopes have a common sequence pattern or motif, as well as MHC allele specificity determining subpatterns.

   To make useful, informative epitope prediction, epitope physicochemical properties are also used, such as exposed surface, accessibility, flexibility, hydrophilicity, charge, number of proline residues, the proximity of the segment towards the C- or N-terminal of the protein, etc. Due to the enormous number of physicochemical properties that are associated with epitopes, simpler quantitative descriptors of amino acid properties are sometimes used to simplify computation.

   Techniques, such as binding motifs, quantitative matrices (QM), virtual matrices, machine learning algorithms (ANN, HMM, SVM), evolutionary algorithms, linear programming, etc. are used to identify the binding peptide. They all have their relative advantages and disadvantages. For example, in a comparative study, Yu et al. suggested that motifs give the most accurate MHC-peptide binding predictions with a limited dataset, but as the data volume increases, machine learning predictions become more reliable.

2. **Structure-based epitope prediction**

   The structure-based prediction model bases on 3D protein structure to screen potential binders. Structural similarity between query protein and template proteins are used to predict epitopes of interest.

3. **Hybrid prediction methods: combining sequential with structural analysis**

   Given the poor performance of epitope predictors based on sequence or structure

analysis, it is clear that any single method cannot accurately predict epitopes. Consequently, some researchers turned to building predictive methods taking advantage of both sequential and structural information. For example, a new method which integrates 3D protein structure with physicochemical properties of amino acids using machine learning methods like Hidden Markov Model (HMM), supporting vector machine (SVM), ANN, etc. improved the prediction precision to a though small but significant degree. Like structural-based approach, its further development is hampered by the limited availability of 3D structure data of antigens and true negative datasets, both to construct better predictors and evaluate the algorithms. There is also the possibility of false positives because different antibodies have overlapping binding sites.

METHODS

1. **ProPred:** predicting binders of 51 HLA-DR (MHC class II of human) alleles.
2. **Propred1:** binding peptides of MHC class I alleles. Matrix based methods
3. **nHLAPred:** Promiscuous MHC class I restricted T cell epitopes: ANN, QM
4. **CTLPred:** predicting cytotoxic T lymphocyte (CTL) epitopes in an antigenic sequence: SVM, QM, ANN
5. **TAPPred:** predicting TAP binding peptide in a protein
6. **BcePred:** Prediction of linear B-cell epitopes, using physico-chemical properties
7. **ABCPred:** predict B cell epitope(s) in an antigen sequence, using artificial neural network
8. **Pcleavage:** SVM based method for Proteosome cleavage prediction
9. **MMBpred:** predict mutated high affinity and promiscuous MHC class-I binding peptides from protein sequence
10. **HLA-DR4Pred:** an SVM and ANN based HLA-DRB1*0401(MHC class II alleles) binding peptides prediction method

RELEVANCE:

The implication of epitope prediction in both public health and basic scientific research is vast. It is applicable to all epitope-related research, such as discovery of peptide candidate for subunit vaccines, autoimmune diseases study, allergy treatment, protein structural study, experiment design, etc. Developing epitope predictive methods and software to identify and map potential epitopes from an antigen protein is vital to contest the immune and infectious diseases. Drug development is the major financial drive for epitope prediction. Epitope-based vaccines have been shown to have promising results and confer protection to animal models in clinical trials, supporting the prophylactic, therapeutic and protective effects of these vaccines. The advantages of subunit vaccines

over other types of vaccines are pronounced. Therefore, huge resources are being channelled into developing subunit vaccines against important, intractable diseases such as cancer, HIV/AIDS, HCV and many other infectious, viral and immune diseases.
However, despite its huge implications in public health, security and scientific arena, epitope prediction tools may be abused by terrorists to make biochemical weapons, and accelerate pathogen evolution and mutation. Another concern is that the application of epitope predictive software in discovering epitopes bias subsequent predictors, as researchers would normally narrow peptide targets by predicting possible epitopes first and then conduct experiments to discover epitopes, which in turn will be analysed to develop other epitope predictive software.

4. Prediction based on signal sequences:

Protein localization is important as protein function may be localized to specific areas inside the cell or within cellular organelles. These bioinformatics programs and databases contain information and are able to predict where a protein may be localized based on signal sequences or localization sequences contained within the protein. Methods involving the recognition of N-terminal signal sequences; as the strong biological implication because the signal sequence specifying the cellular location of a protein is located at the N-terminus (Emanuelsson et al., 2000 and Reczko and Hatzigerrorgiou, 2004). However, it is difficult to recognize underlying features from a highly diverged signal sequence and to vectorize those features.

METHODS:

pTARGET: (Guda and Subramaniam, 2005) uses amino acid composition and localization-specific Pfam domains to assign a eukaryotic protein to one of nine localization sites.
SecretomeP: (Bendtsen et al, 2004) predicts eukaryotic proteins which are secreted via a non-traditional secretory mechanism.
SignalP: (Bendtsen et al, 2004) predicts traditional N-terminal signal peptides in both prokaryotic and eukaryotic proteins.
TargetP: (Emanuelsson et al, 2000) predicts the presence of signal peptides, chloroplast transit peptides, and mitochondrial targeting peptides for plant proteins, and the presence of signal peptides and mitochondrial targeting peptides for eukaryotic proteins.
ChloroP: Chloroplast transit peptides and their cleavage sites in plant proteins

5. Prediction based on Motifs:

The rapid increase in genomic information requires new techniques to infer protein function and predict protein-protein interactions. Bioinformatics identifies modular

signalling domains within protein sequences with a high degree of accuracy. In contrast, little success has been achieved in predicting short linear sequence motifs within proteins targeted by these domains to form complex signalling networks. Predictions from database searches for proteins containing motifs matching two different domains in a common signaling pathway provide a much higher success rate. This technology facilitates prediction of cell signalling networks within proteomes, and could aid in the identification of drug targets for the treatment of human diseases.
Techniques used for finding motifs in given protein sequences:

MEME: tool for discovering motifs in a group of related DNA or protein sequences.

Prosite: This program allows to scan a protein sequence (either from Swiss-Prot or TrEMBL or provided by the user) for the occurrence of patterns and profiles stored in the PROSITE database, or to search protein databases with a user-entered pattern.

PRINTS: is a compendium of protein fingerprints. A fingerprint is a group of conserved motifs used to characterise a protein family; its diagnostic power is refined by iterative scanning of a SWISS-PROT/TrEMBL composite. Usually the motifs do not overlap, but are separated along a sequence, though they may be contiguous in 3D-space.

METHODS:

Pseapred: prediction of secretory proteins of *P.falciparum* method employs MAST technique along with PSI-BLAST and PSSM.

TBpred: prediction server that predicts four subcellular localization (cytoplasmic, integral membrane, secretory and membrane attached by lipid anchor) of mycobacterial proteins .It is SVM based method that exploits different features of protein such as amino acid composition, dipeptide composition and position specific scoring matrix (PSSM). Along with SVM other techniques like profile HMM and MEME/MAST motif based studies were also applied. Moreover a hybrid approach combining the PSSM based SVM model and the MEME/MAST model has been incorporated.

6. Prediction based on Domains:

Protein domain prediction is important for protein structure prediction, structure determination, function annotation, mutagenesis analysis and protein engineering.
Protein domains are structural, functional and evolutionary units of proteins. The prediction of domains from sequence information can improve tertiary structure prediction; enhance protein function annotation, aid structure determination and guide

protein engineering and mutagenesis.
The identification of domains within a protein sequence is an important precursor for a range of methods. Protein structural determination method such as X-ray crystallography and NMR has size limitations which limit their use - they are often employed more successfully when solving smaller domain units rather than whole chains.

METHODS:

MITPred: method for predicting the proteins which are destined to localize in mitochondria. In this method Domain search technique is also employed using my HMMER (hidden Markov Models based search) along with BLAST and SVM.

RELEVANCE:
Domains provide one of the most valuable information for the prediction of protein structure, function, evolution and design. Accurate prediction of domain boundaries forms a basis of many types of protein research. New proteins such as chimeric proteins can be created as they are composed of multifunctional domains (Suyama & Ohara, 2003). The search method for templates used in comparative modeling can also be optimized by the delineation of domain boundaries (Contreras-Moreira & Bates, 2002). As for threading methods, the domain boundary prediction can improve its performance by enhancing the signal-to-noise ratio (Wheelan et al., 2000). Accurate identification of domain boundaries for homologous domains plays a key role for reliable multiple sequence alignment (Gracy & Argos, 1998).

7. Prediction based on Profiles:
Classic profile-based prediction worked well for early single-issue, in-order execution processors, but fails to accurately predict the performance of modern processors. The major reason is that modern processors can issue and execute several instructions at the same time, sometimes out of the original order and cross the boundary of basic blocks.
Prosite is a method of determining what is the function of uncharacterized proteins translated from genomic or cDNA sequences. It consists of a database of biologically significant sites and patterns formulated in such a way that with appropriate computational tools it can rapidly and reliably identify to which known family of protein (if any) the new sequence belongs.
A profile, or weight matrix, is a table of position-specific amino acid weights and gap costs. These numbers (also referred to as scores) are used to calculate a similarity score for any alignment between a profile and a sequence, or parts of a profile and a sequence. An alignment with a similarity score higher than or equal to a given cut-off value

constitutes a motif occurrence. As with patterns, there may be several matches to a profile in one sequence, but multiple occurrences in the same sequences must be disjoint (non-overlapping) according to a specific definition included in the profile.

METHODS:

TBpred: prediction server that predicts four subcellular localization (cytoplasmic, integral membrane, secretory and membrane attached by lipid anchor) of mycobacterial proteins. It is SVM based method that exploits different features of protein such as amino acid composition, dipeptide composition and position specific scoring matrix (PSSM). Along with SVM other techniques like profile HMM and MEME/MAST motif based studies were also applied. Moreover a hybrid approach combining the PSSM based SVM model and the MEME/MAST model has been incorporated.

PPrint: is a web-server for predicting RNA-binding residues of a protein. The prediction is done by SVM model trained on PSSM profile generated by PSI-BLAST search of 'nr' protein database.

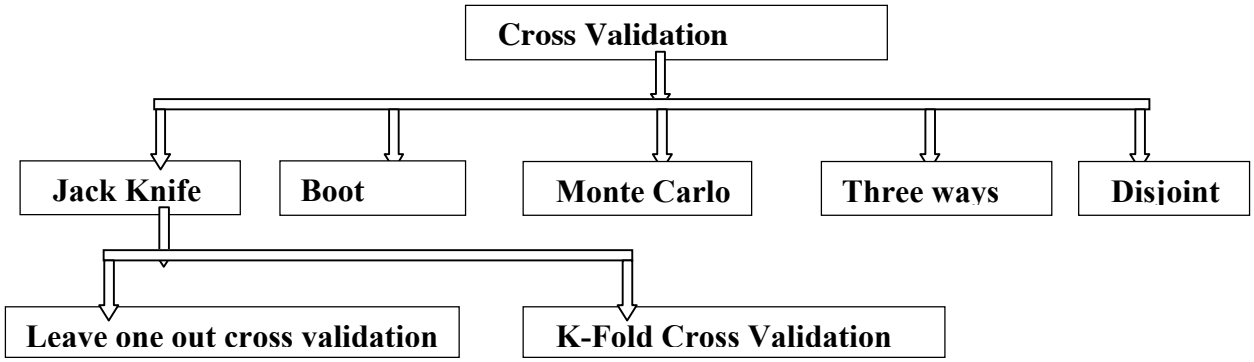
PFMpred: Predicting mitochondrial proteins of *P.falciparum*

ESLPred2: Prediction of subcellular localization of eukaryotic proteins

Evaluation of Bioinformatics Methods

Cross-Validation Technique

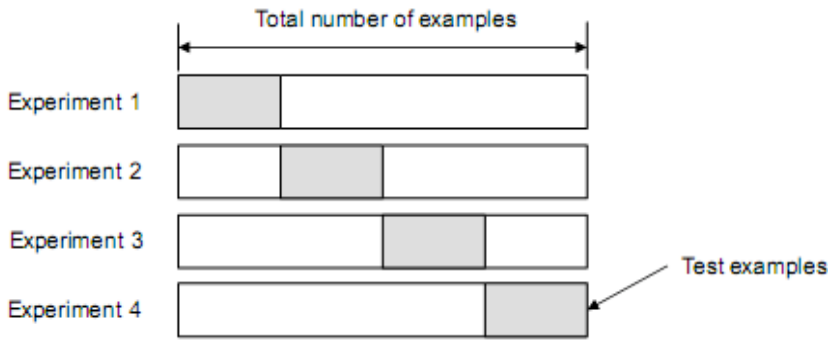
Cross-validation is a statistical method for validating a predictive model. Subsets of the data are held out, to be used as validating sets, a model is fit to the remaining data (a training set) and used to predict for the validation set. Averaging the quality of the predictions across the validation sets yields an overall measure of prediction accuracy.
In cross-validation, the original data set is partitioned into smaller data sets. The analysis is performed on a single subset, with the results validated against the remaining subsets. The subset used for the analysis is called the "training" set and the other subsets are called "validation" sets (or "testing" sets).



Jack Knife Test

Jackknifing, which is similar to bootstrapping, is used in statistical inferencing to estimate the bias and standard error in a statistic, when a random sample of observations is used to calculate it. The basic idea behind the jackknife estimator lies in systematically recomputing the statistic estimate leaving out one observation at a time from the sample set. From this new set of "observations" for the statistic an estimate for the bias can be calculated and an estimate for the variance of the statistic.

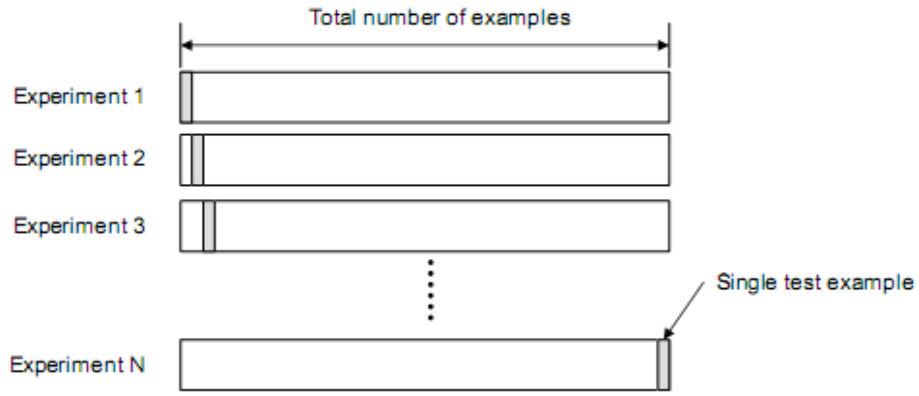
K-fold Cross-validation- For each of K experiments, use K-1 folds for training and a different fold for Testing .This procedure is illustrated in the following figure for K=4



- Advantage of K-Fold Cross validation is that all the examples in the dataset are eventually used for both training and testing.
- Disadvantage of this method is that the training has to be completed *k* times, meaning it takes *k* times as much computation time

Leave-one Out Cross-validation- Leave-one-out is the degenerate case of K-Fold Cross Validation, where K is chosen as the total number of examples.

- For a dataset with N examples, perform N experiments
- For each experiment use N-1 examples for training and the remaining example for testing.



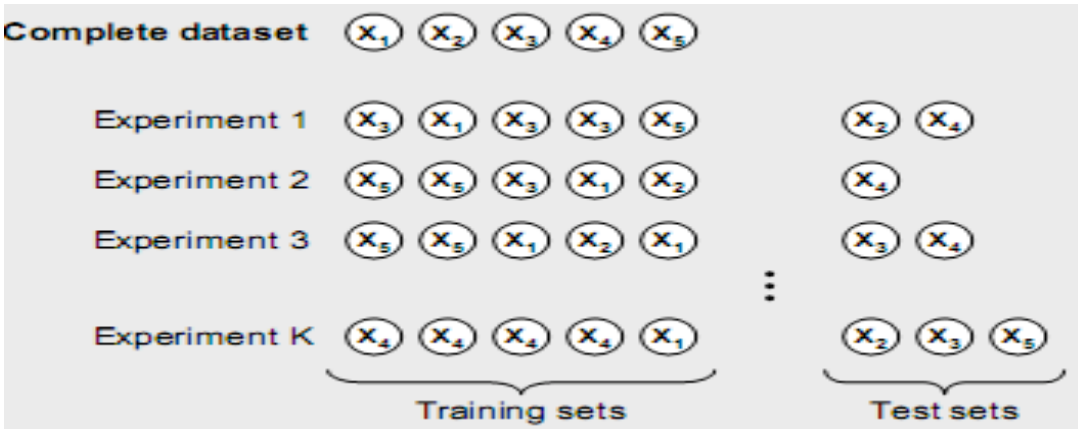
<u>Advantage</u>: Makes best use of the data
Involves no random sub sampling
<u>Disadvantage:</u> Very computationally expensive and stratification is not possible.

Bootstrapping Technique

Bootstrapping technique is a statistical method for estimating the sampling distribution of an estimator by sampling with replacement from the original sample, most often with the purpose of deriving robust estimates of standard errors and confidence intervals of a population parameter like a mean, median, proportion, odds ratio, correlation coefficient or regression coefficient. It is often used as a robust alternative to inference based on parametric assumptions when those assumptions are in doubt, or where parametric inference is impossible or requires very complicated formulas for the calculation of standard errors.

Sample a dataset of n instances n times with replacement to form a new dataset of n instances. Use this data as the training set. The remaining examples that were not selected for training are used for testing .Randomly select (with replacement) N examples and use this set for training. The remaining examples that were not selected for training is used for testing .This value are likely to change from fold to fold.

Repeat this process for a specified number of folds (K).



Monte Carlo Method

Monte Carlo methods are a class of computational algorithms that rely on repeated random sampling to compute their results. This method often used when simulating physical and mathematical systems. This can be loosely described as a statistical method used in simulation (a method that utilizes sequences of random numbers as data) of data.

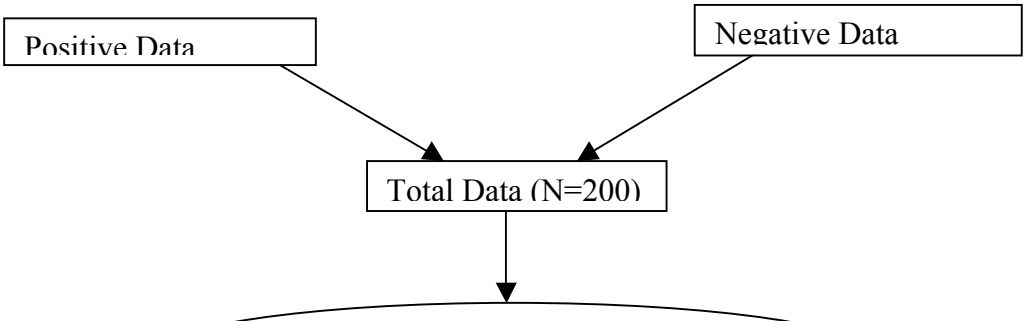
Monte Carlo methods are used to solve various problems by generating suitable random numbers and observing that fraction of the numbers obeying some property or properties. The method is useful for obtaining numerical solutions to problems which are too complicated to solve analytically.

As this method is mainly depend upon random number. So, random number is unique every time. For example a dataset of 200 sequences generate random number (24, 19, 74, 38, 45, 38, 45, 38, 45, 38, 45) .Here the number 38 and 45 repeat many times. This will unnecessarily waste time and give bias model that is not accurate.

Advantage: As number of iteration is better will be the result. For example 10000 iterations give more accurate result as compared to 100 iterations.

Disadvantage: Like any other statistical methods any bias in random number generator will affect the results.

If the model develop during training is wrong, the result may be wrong.



Flow Chart shows the Stepwise procedure of Monte Carlo

NOTE: The tie between the bootstrap and Monte Carlo simulation of a statistic is obvious: Both are based on repetitive sampling and then direct examination of the results. A big difference between the methods, however, is that bootstrapping uses the original, initial sample as the population from which to resample, whereas Monte Carlo simulation is based on setting up a data generation process (with known values of the parameters). Where Monte Carlo is used to test drive estimators, bootstrap methods can be used to estimate the variability of a statistic and the shape of

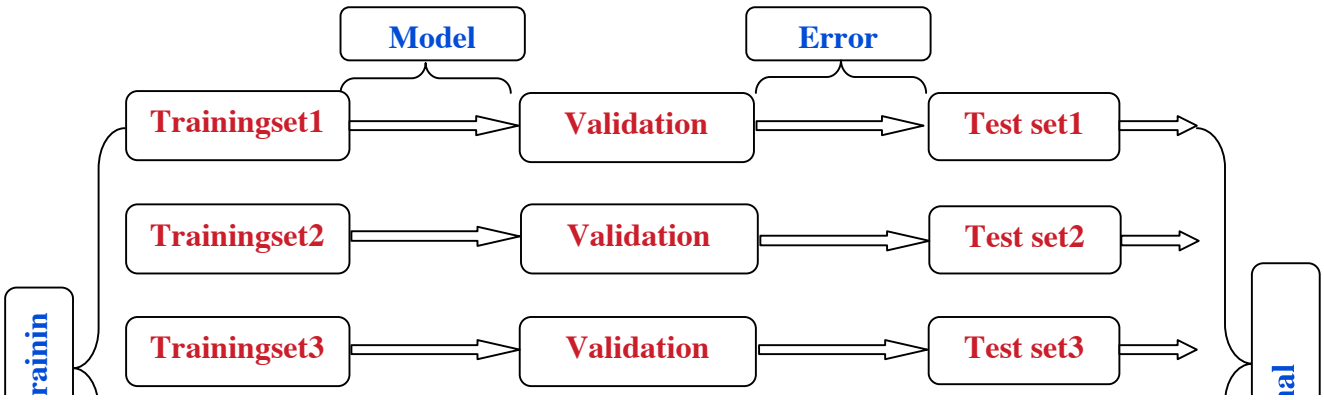
Three Way Split Technique

If model selection and true error estimates are to be computed simultaneously, the data needs to be divided into three disjoint sets.

Training set: A set of examples used for learning: to fit the parameters of the classifier.

Validation set: A set of examples used to tune the parameters of a classifier.

Test set: A set of examples used only to assess the performance of a fully-trained classifier.



Flow Chart shows the Stepwise procedure of three way split

PROCEDURE OUTLINE:

1. Divide the available data into training, validation and test set
2. Select architecture and training parameters
3. Train the model using the training set
4. Evaluate the model using the validation set
5. Repeat steps 2 through 4 using different architectures and training parameters
6. Select the best model and train it using data from the training and validation sets
7. Assess this final model using the test set

Dis-Joint Test

Two sets are said to be **disjoint** if they have no element in common. eg- A={1, 2, 3} and B={4, 5, 6} are disjoint sets. This definition can be extends to any collection of sets. A collection of sets is pairwise disjoint or mutually disjoint. eg- Set A={1, 2}, Set B = {2, 3} and Set C= {3, 1} the intersection of the collection A, B and C is empty, so this is mutually disjoint set but the collection is not pairwise disjoint. In fact, there are no two disjoint sets in the collection.

Criteria using disjoint sets

Number of element/sequences in each set is at least 30.

Set must be pairwise disjoint set otherwise there is bias during training that will result in over prediction.

It is important that the test set is not used in any way to create the classifier.

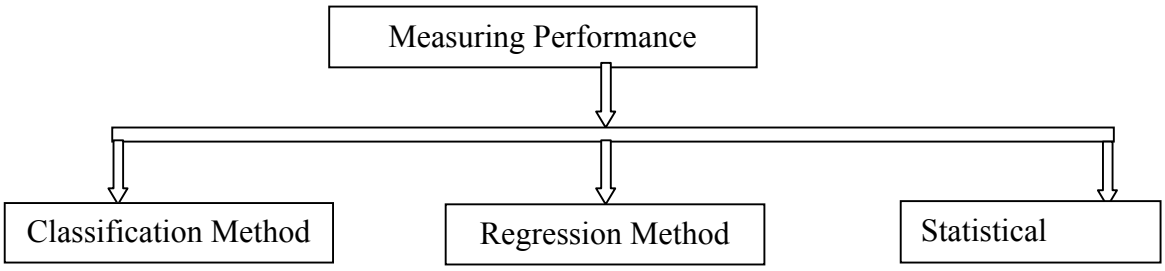
Procedure Outline:

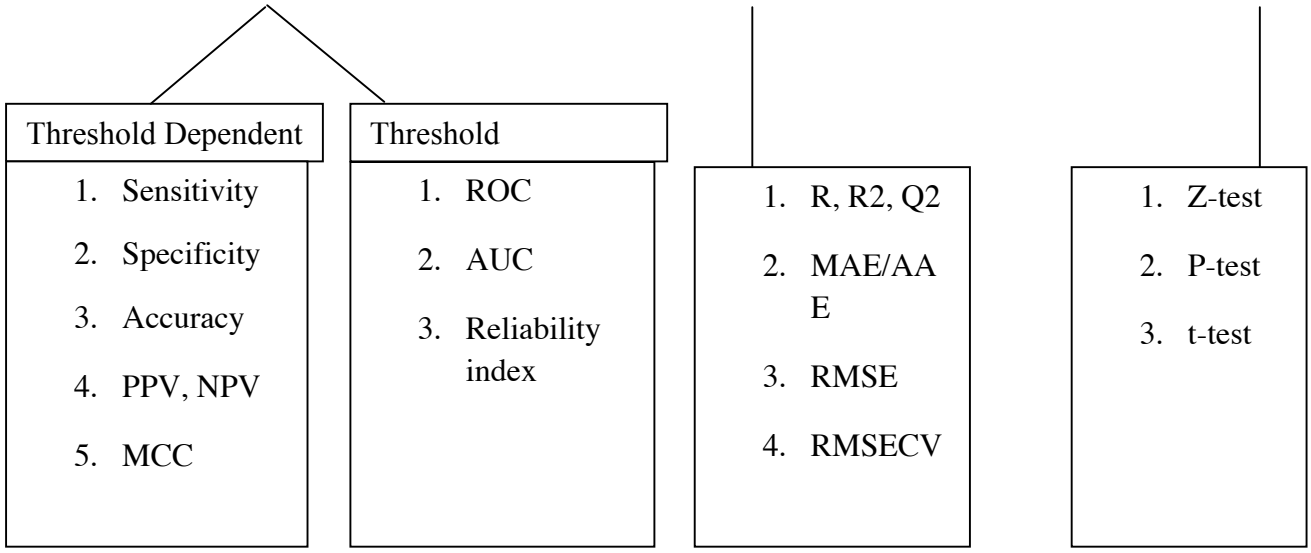
1. Make Positive and Negative datasets in two files. eg- N number sequences for positive and N number for negative sequences.
2. Combine these two file in two a single file.eg- N+N=2N

Non-redundant Five-fold Cross-validation

Ideally sequence in dataset should have minimum sequence similarity (e.g., less than 30% in case of proteins) but it decrease size of dataset significantly. The performance of SVM model directly proportional to size of dataset used for training. We can use non-redundant five-fold cross validation technique, where sequences in dataset were clustered based on sequence similarity. These clustered were divided into five sets; it means all sequences of a cluster were kept in one set. Thus no two sets have similar sequences; it means sequences in training and testing sets have no sequence similarity. We can make clusters using Blastclust and CD-HIT even blastall may also use for this purpose. By using this technique we make non-redundant dataset without decreasing dataset size.

Measuring Performance





		Actual		
		Positive	Negative	
Predicted	Positive	TP	FP	**PPV**
	Negative	FN	TN	**NPV**
		Sensitivity	**Specificity**	

Figure: Criteria of classification of a prediction into TP, TN, FP

Threshold Dependent Parameters

Example: 203 people were examined for checking the probability of lung cancer

P r	**Actual**

		Positive(sick)	Negative (Healthy)	
	Positive (Sick)	TP=2	FP=18	**PPV** =2 / (2 + 18) =10%
	Negative (Healthy)	FN=1	TN=182	**NPV** =182 / (1 + 182) =99.5%
		Sensitivity =2/(2+1) =66.67%	**Specificity** =182/18+182 =91%	

- True positive (TP) : Sick people correctly diagnosed as sick
- False positive (FP) : Healthy people wrongly identified as sick
- True negative (TN) : Healthy people correctly identified as healthy
- False negative (FN) : Sick people wrongly identified as healthy

Sensitivity or percentage coverage of positive is the percentage of positive example predicted as positive.

$$\text{Senstivity} = \frac{\text{TP}}{\text{TP} + \text{FN}} \times 100$$

A sensitivity of 100% means that the test recognizes all sick people as such. Thus in a high sensitivity test, a negative result is used to rule out the disease.

Sensitivity alone does not tell us how well the test predicts other classes (that is, about the negative cases). In the binary classification, as illustrated above, this is the corresponding specificity test, or equivalently, the sensitivity for the other classes.

Specificity or percentage coverage of negative is the percentage of negative examples predicted as negative.

$$\text{Specifity} = \frac{\text{TN}}{\text{TN} + \text{FP}} \times 100$$

A specificity of **100%** means that the test recognizes all healthy people as healthy. Thus a positive result in a high specificity test is used to confirm the disease. The maximum is trivially achieved by a test that claims everybody healthy regardless of the true condition.

Therefore, the specificity alone does not tell us how well the test recognizes positive cases. We also need to know the senstivity of the test to the class, or equivalently, the specificities to the other classes.

Probability of Positive Correct Prediction (PPV)
The positive predictive value is the proportion of patients with positive test results who are correctly diagnosed.

$$\text{PPV} = \frac{\text{TN}}{\text{TP} + \text{FP}} \times 100$$

Probability of Negative Correct Prediction (NPV)
The negative predictive value is the proportion of patients with negative test results who are correctly diagnose.

$$\text{NPV} = \frac{\text{TN}}{\text{TN} + \text{FN}} \times 100$$

Accuracy is the degree percentage of correctly predicted examples (both correct positive and correct negative prediction).

$$\text{Accuracy} = \frac{\text{TP} + \text{TN}}{\text{TP} + \text{TN} + \text{FP} + \text{FN}} \times 100$$

= (2+182/2+182+18+1) _ 100 = 90.64%

Matthews Correlation Coefficient is used in machine learning as a measure of the quality of binary (two class) classifications. It takes into account true and false positives and negatives and is generally regarded as a balanced measure which can be used even if the classes are of very different sizes.
It returns a value between -1 and +1. A coefficient of +1 represents a perfect prediction, 0 an average random prediction and -1 an inverse prediction.
The Matthews Correlation Coefficient is generally regarded as being one of the best such measures. In this equation,

$$\text{MCC} = \frac{(\text{TP} \times \text{TN}) - (\text{FP} \times \text{FN})}{\sqrt{(\text{TP} + \text{FP})(\text{TP} + \text{FN})(\text{TN} + \text{FP})(\text{TN} + \text{FN})}} \times 100$$

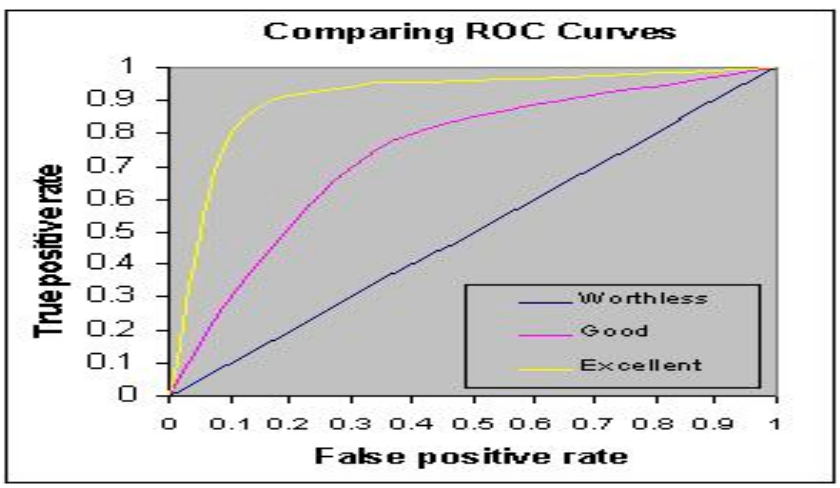
= (2_182) - (18_1)/sqrt ((2+18) _ (2+1) _ (118+18) _ (118+1)
= 346/sqrt (971040)
= 0.371

If any of the four sums in the denominator is zero, the denominator can be arbitrarily set to one; this results in a Matthews Correlation Coefficient of zero, which can be shown to

be the correct limiting value.

Threshold Independent Parameter

Receiver operating characteristic (**ROC**) or simply **ROC curve** is a graphical plot of the sensitivity vs. (1- specificity) for a binary classifier system as its discrimination threshold is varied. The ROC can also be represented equivalently by plotting the fraction of true positives (TPR = true positive rate) vs. the fraction of false positives (FPR = false positive rate) also known as a Relative Operating Characteristic curve, because it is a comparison of two operating characteristics (TPR & FPR) as the criterion changes.
ROC analysis provides tools to select possibly optimal models and to discard suboptimal ones independently from (and prior to specifying) the cost context or the class distribution. ROC analysis is related in a direct and natural way to cost/benefit analysis of diagnostic decision making. ROC analysis has more recently been used in medicine, radiology, psychology, and other areas for many decades, and it has been introduced relatively recently in other areas like machine learning and data mining.
AUC: The area under the ROC curve, is called Area under the curve (**AUC**), or A' (pronounced "a-prime"). If AUC value is more than 0.5 then our model is working well otherwise it's a worse model.



Reliability Index
Reliability index is a simple indication of level of certainty in the prediction. This RI calculated by the following given equation-

$$RI = \begin{cases} INT\,(diff \times 5/3 + 1 & if\ 0 \le diff < 4, \\ 5 & if\ diff \ge 4 \end{cases}$$

RI is used in multiclass classification study. Assignment of RI to each sequence based

upon the difference of highest and second highest score of various 1-vs-rest SVMs in multi-class classification.

Regression Method

Regression/Real Value: We used machine learning techniques in regression/real-value prediction. In this we predict real value as melting point, boiling point, IC_{50}, K_d, EC_{50} etc. These are the parameter which gives explanation how good predicted values are good in compare to its real value. To access model performance and provide statistically meaningful data, we can calculate different statistical parameters. Here I am giving formulas using melting point (MP) as an example.

Actual MP (MP^{act})	**Predicted MP** (MP^{pred})
12.5	14.0
67.0	71.3
71.2	68.7
115.9	121.0
32.7	29.8
45.7	49.3
79.8	76.8
127.3	125.1
57.6	50.2
37.2	33.8
$\sum(MP^{act}) = 646.90$	$\sum(MP^{pred}) = 640.0$
$\sum(MP^{act})^2 = 53580.21$	$\sum(MP^{pred})^2 = 53169.64$

$$\sum MP^{act} MP^{pred} = 53297.66$$

Mean ($\overline{MP}$): The *arithmetic mean* is the "standard" average, often simply called the "mean".

[redacted]

So here mean of actual

$\overline{MP}$ = 12.5+67.0+71.2+115.9+32.7+45.7+79.8+127.3+57.6+37.2/10

= 646.90/10 = 64.69

Similarly $\overline{MP^{pred}}$ = 14.0+71.3+68.7+……../10

= 640.00/10 = 64.0

Pearson's correlation/Sample correlation (R): In general statistical usage, *correlation* refers to the departure of two random variables from independence. **R** is the Pearson's correlation coefficient of actual and predicted value, this give idea about the performance of machine learning techniques.

[redacted]

R = 53297.66 – 646.90*646.0/sqrt (53580.21 - 646.90^2)*(53169.64 – 646.00^2)
= 11896.06/11968.56
= 0.994

Where n is the size of test set, MP^{pred} is the predicted melting point and MP^{act} is the actual melting points. Value of R always ranges from -1 to +1 negative. Negative value of R shows that there is inverse relationship within actual and predicted value; while positive value of R show that here positive relationship within actual and predicted value. If R = 0 then it's totally random prediction.

Coefficient of determination (R^2): Coefficient of determination is the statistical parameter for proportion of variability in model.

[redacted]

Sum of square of errors (SSE) = $\sum_{i=1}^{n} \left(MP^{act} - MP^{pred}\right)^2$

Sum of square of total (SST) = $\sum_{i=1}^{n} \left(MP^{act} - \overline{MP}\right)^2$

Where MP^{pred} is the predicted melting point and MP^{act} is the actual melting points $\overline{MP}$ is the mean of MP^{act}.

R2 = 1 – (SSE/SST)
=1 – (154.53/11732.249) = 0.87

The coefficient of determination is also the arithmetic average of all M folds run. Value of R^2 always ranges within 0 to 1. Its value gives idea how these actual values are related with predicted value. Higher values of R^2 show that here linear relationship within actual and predicted and lower value shows that non-linear relationship

Q^2 is another very important statistical parameter for the determination of variability in model.

[redacted]

If value is more 0.5 then models performance is good.

RMSE is the root mean squared error of the predictions calculated according

[redacted]

= sqrt (154.53/10)

= 3.931

Where **n** is the size of test set, MP^{act} is the actual melting point and MP^{pred} is the predicted melting point by different machine learning techniques. Like mean absolute error it's also give idea how our predicted melting point is for away from actual melting points.

MAE/AAE is mean of absolute errors within actual and predicted value

[redacted]

= 1/10* (| 12.5-14.0| + |67.0-71.3|+ …..)

= 3.59

Its gives idea how our predicted value are for away from experimentally calculated melting point. Where n is the size of test set, MP^{act} is the actual melting point and MP^{pred} is the predicted melting point by different machine learning techniques.

RMSECV is the aggregate root mean squared error of the cross-validation. For an M fold cross-validation, it is defined as

[redacted]

Statistical Method

z-Test- The Z-test compares sample and population means to determine if there is a significant difference.

It requires a simple random sample from a population with a Normal distribution and where the mean is known.

Calculation The z measure is calculated as:

$$Z = (x - m) / SE$$

where **x** is the mean sample to be standardized
m is the populations mean, **SE** is the standard error of the mean.

SE = s / sqrt(n)

where **s** is the population standard deviation, **n** is the sample size

The z value is then looked up in a z-table. A negative z value means it is below the population mean (the sign is ignored in the lookup table).

- The Z-test is typically with standardized tests, checking whether the scores from a particular sample are within or outside the standard test performance.
- The z value indicates the number of standard deviation units of the sample from the population mean.

Note: z-test is not the same as the z-score, although they are closely related.

t-Test- The t-test assesses whether the means of two groups are statistically different from each other. This analysis is appropriate whenever you want to compare the means of two groups.

$$t - value = \frac{Difference\ between\ group\ means}{Variability\ of\ groups}$$

$$= \frac{\overline{X_T} - \overline{X_C}}{SE(\overline{X_T} - \overline{X_C})}$$

$$SE(\overline{X_T} - \overline{X_C}) = \sqrt{\frac{var_T}{n_T} + \frac{var_C}{n_C}}$$

$$t = \frac{\overline{X_T} - \overline{X_C}}{\sqrt{\frac{var_T}{n_T} + \frac{var_C}{n_C}}}$$

In the formula of t-test numerator is difference between the means and denominator is standard error of the difference between mean ,which is calculated by the variances for each group and divide it by the number of people in that group. We add these two values and then take their square root.

The t-value will be positive if the first mean is larger than the second and negative if it is smaller. Once you compute the t-value you have to look it up in a table of significance to test whether the ratio is large enough to say that the difference between the groups is not likely to have been a chance finding. To test the significance, you need to set a risk level (called the alpha level).

p-Test: Hypothesis Tests About a Proportion

In p-test we would like to test the following three null hypotheses about a population

proportion p

1. Ho: p <= P
2. Ho: p >= P
3. Ho: p = P

We can test each claim simultaneously with a sample proportion m / n, where m is the number of favorable (or "Yes") responses and n is the random sample size.
If m / n are too large, then we must reject the first null hypothesis Ho: p <= P.
If m / n are too small, then we reject the second null hypothesis. Ho: p >= P
If m / n are either too large or too small, then we reject the third null hypothesis. Ho: p = P

Once again, we conduct the tests with the use of the test statistic. If the population is considered "large," then we define the test statistic by
If the population is of a smaller, finite size N (so that the sample size n is more than 5% of the entire population), then we define the test statistic by

$$x = (m / n - P) / \text{Sqrt}[P(1 - P) / n].$$

$$x = (m / n - P) / [\ \text{Sqrt}[\ P(1 - P) / n]\ \text{Sqrt}[\ (N - n) / (N - 1)\ ]\ ]$$

Commonly used Bioinformatics Tools

This chapter describes commonly used computational techniques like machine learning. The aim of this chapter is not to describe theory of these methods. Instead we have describes how to use these programs. We have describes these methods in short and

simple words, so begineers may use these tools. The detail description of these programs is available from their manual or web site. Following are commonly used tools, particularly our group is using them to build new tools.

Support Vector Machine: How to use SVM^{light}

SVM is frequently used in bioinformatics for classifying proteins, predicting structures, epitop prediction etc. One of the major advantages of SVM over other machine learning techniques is that it can be trained on small data set with minimum over-optimization. SVM^{light} is an implementation of Support vector Machines (SVMs) in C. SVM^{light} is an implementation of Vapnik's Support Vector Machine for the problem of pattern recognition, for the problem of regression, and for the problem of learning a ranking function. The algorithm has scalable memory requirements and can handle problems with many thousand of support vectors efficiently. The software also provides methods for assessing the generalization performance efficiently. It includes two efficient estimation methods for both error rate and precision/recall.

How to use

SVM^{light} consists of a learning module (svm_learn) and a classification module (svm_classify). The classification module can be used to apply the learned model to new examples. See also the examples below for how to use svm_learn and svm_classify.

Run the svm_learn program with different parameters for better optimization

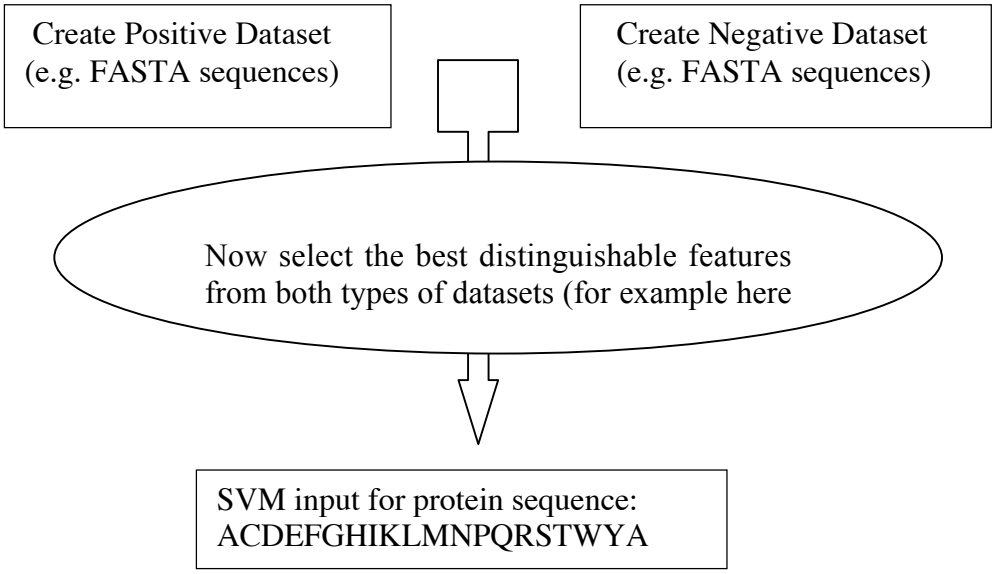
svm_learn [options] example_file model_file
svm_learn program build a model amd these model further use with svm_classify program.

1. By the use of svm_classify we can predict the class of unknown protein, whether it's belongs to positive type or negative type.

   svm_classify [options] example_file model_file output_file

   svm_learn is called with the following parameters:

   svm_learn [options] example_file model_file



SVM input (for Positive sequence)	SVM input (for Negative sequence)
+1 1:10 2:5 3:5 4:5 5:5 6:5 7:5 8:5 9:5 10:5 11:5 12:5 13:5 14:5 15:5 16:5 17:5 18:0 19:5 20:5	-1 1:10 2:5 3:5 4:5 5:5 6:5 7:5 8:5 9:5 10:5 11:5 12:5 13:5 14:5 15:5 16:5 17:5 18:0 19:5 20:5

Artificial Neural Network: How to use the SNNS for implementing ANN

ANN is powerful machine learning techniques, commonly used for solving classification problem. They are capable to handle large datasets and non-linear proroblems efficiently. SNNS (Stuttgart Neural Network Simulator) is a software simulator for neural networks on Unix workstations developed at the Institute for Parallel and Distributed High Performance Systems (IPVR) at the University of Stuttgart. The goal of the SNNS project is to create an efficient and flexible simulation environment for research on and

application of neural nets. One of the challenges is to implement SNNS, here we have given an example

Input file in fasta format

Total number of sequence in this file is 78, only 10 are diplayed

An example of sequences in fasta format,

```
>Lec_protein1
ADSGADSGFADSGDAGSFDAGDSGFADSGFADSGDAGSDAGDSGAD
>Lec_protein2
ASKDNAKSNDKJASNDKJANSKDNASKMDKMASNKDNASKJNDKAL
>Lec_protein3
XLKAMSLKXMALKSMXLKASMXLKASMXLMASLXMALSXMLAKS
>Lec_protein4
LJDLKAJSLKDJASLKJDLASJDLAJSLDJASLDLAJSLDKJALSJDLKAJS
>Lec_protein5
JRTLKERJLKTJELRJTLKERJTLKJERLKJTLKERJTLKJERLKTJERLKJT
>Lec_protein6
DLJASLKJDLASJDLKJASLDKJASLDJLASKJDLKJASLDJASLKJDALSK
>Lec_protein7
LASJDLAJSLDJASLJDALSJDLAJSLDJASLJDLASJDLKJASLDJALSJDLK
>Lec_protein8
ENRWMENRMWNERNWERNWERMWEMNRWENRMWENRNWMENRM
>Lec_protein9
NWEMWMENQNEQNEQMNEQMNWMENQWNEQNWEQMWNEMQWN
>Lec_protein10
LKASLKDJASJDLKAJSLDJASLJDLKASJASLJDALKSDLKJALDLKASJDL
```

Input file in SNNS format

In order to generate fixed length pattern from variable length of sequence, we compute amino acid composition. Following is example input SNNS file generated for these sequences where composition is feature. Following is descriptrion

Note that the first 7 lines of the input file. First two lines , followed by to blank line then the number of patterens (78 in this case, since total seqience is 78), number of input units (20 in this case, calculating the amino acid composition) and the out puts (1, one value)

SNNS pattern definition files V4.2

Generated at Sat Aug 27 16:40:25 2005

No. of patterns: 78
No. of input units: 20
No. of output units: 1

Input pattern 1:
0.1 0 0.2 0.2 0 0.1 0 0 0 0 0 0.1 0 0.3 0 0 0 0 0 0
Output pattern 1:
1
Input pattern 2:
0.1 0 0 0.5 0 0 0 0 0.1 0.1 0 0 0 0 0.1 0 0 0.1 0 0
Output pattern 2:
1

Output file of SNNS
The out put file of the SNNS is shown. The result shows the summary of information.
SNNS result file V1.4-3D
Generated at Tue Aug 30 08:58:52 2005

No. of patterns : 26
No. of input units: 20
No. of output units: 1
Startpattern : 1
Endpattern : 26
Input patterns included
Teaching output included
#1.1
0.1 0 0.1 0 0 0 0 0.1 0.1 0.2
0 0 0 0 0 0.1 0.1 0 0 0.2
1
0.64832 ← Out put of SNNS
#2.1
0 0 0.1 0.1 0.3 0.1 0 0 0 0
0 0 0.2 0.1 0 0 0.1 0 0 0
1
0.6276

The outputs of the SNNS are process at different threshold (0.1 to 1), and parameters like

sensitivity, specificity, and accuracy are calculated. The Artificial neural network tries to classify positive from negative examples. For example here we take an example of IgE epitopes and non epitopes. We need a data set of IgE epitope (positive set) and negative set (non epitopes). The Netwok will classify this training set, it will be validated by one set (to stop over fitting) and then tested by the left out testing set. Each set contains equal number of sequence. In five fold cross validation it looks like this,

Training set	Validation set	Testing set
set 1,2,3	set 4	set 5
set 1,4,5	set 3	set 4
set 1,4,5	set 2	set 3
set 3,4,5	set 1	set 2
set 2,3,4	set 5	set 1

Processing of output data

The out put data are processed and interpreted, as shown (Thres=Threshold; Sen=Sensitivity; Spe= Specificity; Acc=Accuracy; PPV=positive prediction value)

Thres	Sen	Spe	Acc	PPV
1.0000	0.0000	0.0000	0.0000	0.0000
0.9000	0.0214	0.9929	0.5071	0.7500
0.8000	0.1429	0.9857	0.5643	0.9091
0.7000	0.2571	0.9571	0.6071	0.8571
0.6000	0.5143	0.8357	0.6750	0.7579
0.5000	0.7214	0.7214	0.7214	0.7214
0.4500	0.8071	0.6000	0.7036	0.6686
0.4000	0.8571	0.4714	0.6643	0.6186
0.3000	0.9571	0.3286	0.6429	0.5877
0.2000	1.0000	0.1000	0.5500	0.5263
0.1000	000	0.0071	0.5036	0.5018

HMMER: Bio-sequences analysis using profile hidden markov models

Introduction

HMMER is a freely distributable implementation of profile HMM software for protein sequence analysis written by Sean Eddy. It is used for sensitive database search using multiple sequence alignments (profile-HMMs) as queries. The profile-HMMs are based on the work of Krogh and colleagues. Basically, we give HMMER a multiple sequence alignment as input; it builds a statistical model called a "hidden Markov model" which you can then use as a query into a sequence database to find (and/or align) additional homologues of the sequence family. HMMER is a console utility ported to every major operating system including different versions of Linux, Windows and Mac OS.

HMMER generally contain following programs ---

hmmalign
: Align sequences to an existing model.

hmmbuild
: Build a model from a multiple sequence alignment.

hmmcalibrate
: Takes an HMM and empirically determines parameters that are used to make searches more sensitive, by calculating more accurate expectation value scores (E-values).

hmmconvert
: Convert a model file into different formats, including a compact HMMER 2 binary format, and ``best effort" emulation of GCG profiles.

hmmemit
: Emit sequences probabilistically from a profile HMM.

hmmfetch
: Get a single model from an HMM database.

hmmindex
: Index an HMM database.

hmmpfam
: Search an HMM database for matches to a query sequence.

hmmsearch
: Search a sequence database for matches to an HMM.

KNN: k-Nearest Neighbor

Memory-Based Learning is a direct descendant of the classical k-Nearest Neighbor (k-NN) approach to classification, which has become known as a powerful pattern classification algorithm for numeric data. In typical NLP learning tasks, however, the focus is on discrete data, very large numbers of examples, and many attributes of differing relevance. Moreover, classification speed is a critical issue in any realistic application of Memory-Based Learning. These constraints demand non-trivial data-structures and speedup optimizations for the core k-NN classifier. Our approach has resulted in an architecture which compresses the typical flat file organization found in straightforward k-NN implementations, into a decision-tree structure. While the decision tree can be used to retrieve the exact k-nearest neighbors (as happens in the IB1 algorithm within TiMBL), it can also be deterministically traversed as in a decision-tree classifier (the method adopted by the IGTREE algorithm). We believe that our optimizations make TiMBL one of the fastest discrete k-NN implementations around.

TiMBL is free software; you can redistribute it and/or modify it under the terms of the GNU General Public License as published by the Free Software Foundation; either version 3 of the License, or (at your option) any later version.

Input file format

```
2.3, 5.6, 8.9, 4.5, 2.6, 1.2, 4.7, 4.1, 8.2, 2.1, 3.2, 0.5, 4.8, 7.1, 2.6, 3.1, 1.3, 2.3, 4.0,
1.5, +1
5.3, 2.6, 6.4, 5.8, 9.7, 2.5, 1.5, 4.3, 1.0, 2.4, 5.0, 1.3, 3.2, 1.0, 2.1, 3.5, 8.8, 9.2, 1.8,
6.7, –1
4.2, 1.3, 2.0, 1.5, 0.6, 7.0, 9.4, 3.3, 1.6, 8.2, 4.9, 7.8, 2.0, 3.4, 2.1, 3.8, 2.1, 6.4, 9.7,
3.4, +1
3.1, 2.5, 3.2, 1.4, 8.0, 2.4, 6.2, 1.3, 4.9, 5.4, 3.1, 8.3, 4.7, 2.3, 4.9, 2.4, 3.1, 8.3, 2.4,
6.7,  –1
5.4, 3.6, 2.8, 3.4, 6.7, 2.4, 1.5, 3.6, 9.8, 7.5, 2.3, 4.6, 1.2, 5.7, 8.9, 3.4, 5.2, 1.3, 4.5,
6.8, +1
```

Output file format

```
2.3, 5.6, 8.9, 4.5, 2.6, 1.2, 4.7, 4.1, 8.2, 2.1, 3.2, 0.5, 4.8, 7.1, 2.6, 3.1, 1.3, 2.3, 4.0,
1.5, +1, 5.8
```

5.3, 2.6, 6.4, 5.8, 9.7, 2.5, 1.5, 4.3, 1.0, 2.4, 5.0, 1.3, 3.2, 1.0, 2.1, 3.5, 8.8, 9.2, 1.8, 6.7, –1, 6.4 4.2, 1.3, 2.0, 1.5, 0.6, 7.0, 9.4, 3.3, 1.6, 8.2, 4.9, 7.8, 2.0, 3.4, 2.1, 3.8, 2.1, 6.4, 9.7, 3.4, +1, 4.3 3.1, 2.5, 3.2, 1.4, 8.0, 2.4, 6.2, 1.3, 4.9, 5.4, 3.1, 8.3, 4.7, 2.3, 4.9, 2.4, 3.1, 8.3, 2.4, 6.7, –1, 6.1
5.4, 3.6, 2.8, 3.4, 6.7, 2.4, 1.5, 3.6, 9.8, 7.5, 2.3, 4.6, 1.2, 5.7, 8.9, 3.4, 5.2, 1.3, 4.5, 6.8, +1, 7.0

Class predicted value

This predicted values use in calculating TP, TN, FP and FN parameters.

TP : True Positive TN : True Negative FP : False Positive FN : False Negative

---

CD-HIT

1. CD-HIT: clustering and comparing large sets of sequences

Introduction

Cd-hit is a fast program for clustering and comparing large sets of protein or nucleotide sequences. The main advantage of this program is its ultra-fast speed. It can be hundreds of times faster than other clustering programs, for example, BLASTCLUST. Therefore it can handle very large databases, like NR. Current CD-HIT package can perform various jobs like clustering a protein database, clustering a DNA/RNA database, comparing two databases (protein or DNA/RNA), generating protein families, and many others.

CD-HIT clusters proteins into clusters that meet a user-defined similarity threshold, usually a sequence identity. Each cluster has one representative sequence. The input is a protein dataset in fasta format and the output are two files: a fasta file of representative sequences and a text file of list of clusters.

Basic command:
cd-hit -i nr -o nr100 -c 1.00 -n 5 -M 2000

cd-hit -i db -o db90 -c 0.9 -n 5, where
db is the filename of input,
db90 is output,
0.9, means 90% identity, is the clustering threshold
5 is the size of word

Choose of word size:
-n 5 for thresholds 0.7 ~ 1.0
-n 4 for thresholds 0.6 ~ 0.7
-n 3 for thresholds 0.5 ~ 0.6
-n 2 for thresholds 0.4 ~ 0.5

CD-HIT-2D

CD-HIT-2D compares 2 protein datasets (db1, db2). It identifies the sequences in db2 that are similar to db1 at a certain threshold. The input are two protein datasets (db1, db2) in fasta format and the output are two files: a fasta file of proteins in db2 that are not similar to db1 and a text file that lists similar sequences between db1 & db2.

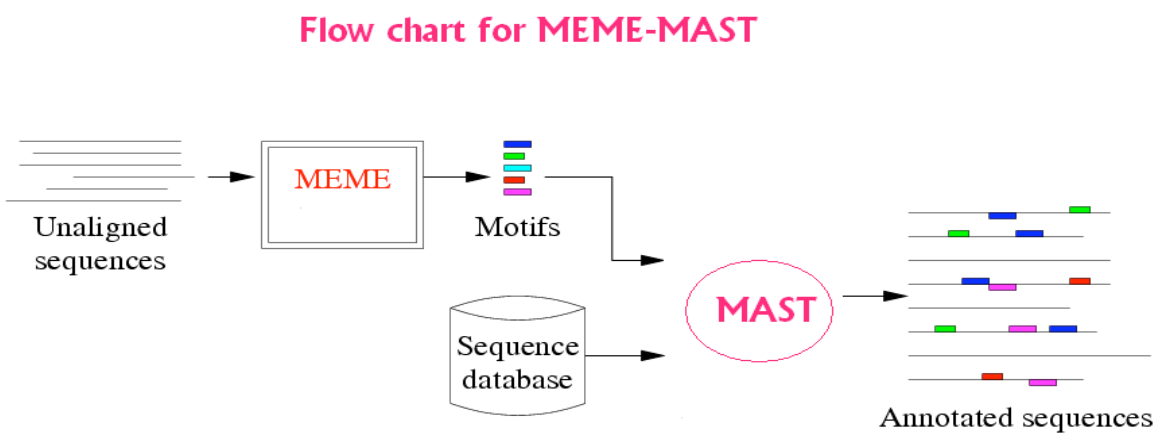
Basic command:
cd-hit-2d -i db1 -i2 db2 -o db2novel -c 0.9 -n 5, where
db1 & db2 are inputs,
db2novel is output,
0.9, means 90% identity, is the comparing threshold
5 is the size of word
Please note that by default, I only list matches where sequences in db2 are not longer than sequences in db1. You may use options -S2 or -s2 to overwrite this default. You can also run command:
cd-hit-2d -i db2 -i2 db1 -o db1novel -c 0.9 -n 5
Choose of word size (same as cd-hit):
-n 5 for thresholds 0.7 ~ 1.0
-n 4 for thresholds 0.6 ~ 0.7
-n 3 for thresholds 0.5 ~ 0.6
-n 2 for thresholds 0.4 ~ 0.5

MEME/MAST

MEME: MEME is a tool for discovering motifs in a group of related DNA or protein sequences.

The MEME Suite software is available for FREE interactive use via the web or you can download it on your local system from http://meme.nbcr.net/meme4_1/meme-download.html web link.

MEME takes as input a group of DNA or protein sequences and outputs as many motifs as requested. MEME uses statistical modeling techniques to automatically choose the best width, number of occurrences, and description for each motif.



Program Execution:

meme meme_input_file (options) > meme_output_file

NUMBER OF MOTIFS
-nmotifs <n> The number of *different* motifs to search for. MEME will search for and output <n> motifs. Default: 1

-evt <p> Quit looking for motifs if E-value exceeds <p>.Default: infinite (so by default MEME never quits before -nmotifs <n> have been found.)

NUMBER OF MOTIF OCCURENCES
-nsites <n>
-minsites <n>
-maxsites <n> the (expected) number of occurrences of each motif. If -nsites is given, only that number of occurrences is tried. Otherwise, numbers of occurrences between -minsites and -maxsites are tried as initial guesses for the number of motif occurrences. These switches are ignored if mod = oops.
Default: -minsites sqrt (number sequences)
-wnsites <n> the weight on the prior on nsites. This controls how strong the bias towards motifs with exactly nsites sites (or between minsites and maxsites sites) is. It is a number in the range [0..1). The larger it is, the stronger the bias towards motifs with exactly nsites occurrences is. Default: 0.8

MOTIF WIDTH

-w <n>
-minw <n>
-maxw <n>

The width of the motif(s) to search for. If -w is given, only that width is tried. Otherwise, widths between -minw and -maxw are tried. Default: -minw 8, -maxw 50 (defined in user.h)

Note: If <n> is less than the length of the shortest sequence in the dataset, <n> is reset by MEME to that value.

MAST: MAST is a tool for searching biological sequence databases for sequences that contain one or more of a group of known motifs.

MAST takes as input a MEME output file containing the descriptions of one or more motifs and searches a sequence database that you select for sequences that match the motifs

mast <meme_output_file> [-d <database>] [optional arguments ...]

<mfile> file containing motifs to use (meme_output_file)

-d database to search with motifs

Quantitative matrix

The contribution of each residue (amino acid) for each position in a polypeptide chain can be calculated with the use of Quantitative matrix. The QM is basically a propensity of each residue at a particular position. There are a number of equations, which can be used for matrix generation. The higher positive score of a residue at a given position means this residue is highly preferred at that position. The higher negative score means that residue is not preferred in peptides at that position. One of the major advantages of QM is that the effect of each residue on specific activity of a peptide can be easily estimated.

Quantitative Matrix: These quantitative based methods consider the contribution of each residue at each position in peptide instead of anchor positions/residues. Quantitative matrices provide a linear model with easy to implement capabilities. Another advantage of using the matrix approach is that it covers a wider range of peptides with binding potential and it gives a quantitative score to each peptide. Their predictive accuracies are considerable.

Equation for Matrix Generation: There are a number of equations which can be used for matrix generation.

A few of which are as follows

Q (i,r) = **P**(i,r) – **N**(i,r) (1)

P (i,r) = **E** i,r / **NP** i,r (2)

N (i,r) = **A** i,r / **NN** i,r (3)

Where, Q(i,r) is the weight of any residue r at position 'i' in the matrix. 'r' can be any natural amino acid and the value of 'i' can vary from 1 to 15. P(i,r) and N(i,r) is the probability of residue 'r' at position 'i' in positive and negative peptides respectively. E i,r and A i,r is number residue 'r' at position 'i' in positive and negative peptides respectively, and NP i,r is the number of positive peptides and NN i,r is the number of negative peptides having residue 'r' at position 'i'.

Example:

Generation of Quantitative matrices: The quantitative matrices consist of a table having the sequence weight

Frequencies of each of the 21 amino acids (including "X") at each position in the dataset of MHC binders divided by the corresponding expected frequency of that amino acid in the non-binders dataset. The MHC binder's datasets for each MHC allele are generated by obtaining MHC binders of 9 amino acids from MHCBN database. The equal number of the non-binders is also obtained from the same database (if available) otherwise the 9-mer peptides are randomly chosen from the SWISS-PROT database. The quantitative matrices are addition matrices where the score of a peptide is calculated by summing up the scores of each residue at specific position along peptide sequence. For example, the score of peptide "ILKEPVHGV" is calculated as follows.

Score= I(1)+L(2)+K(3)+E(4)+P(5)+V(6)+H(7)+G(8)+V(9)

The peptides with score more than the cutoff score at a particular threshold are predicted as MHC binders. A fewmatrices are also obtained from literature (BIMAS and ProPred1).These matrices are mostly multiplication matrices. The score of the peptide is calculated as follows: e.g. "ILKEPVHGV"

Peptide score=I(1)*L(2)*K(3)*E(4)*P(5)*V(6)*H(7)*G(8)*V(9)

Protein General Modules

In this chapter we have described the small programs developed at our group; these programs can be used as building block to develop complex prediction modules. The question arises how it is different then existing software libraries or modules like BioPERL. InBioPER or similar packages one need to have knowledge of computer programming in order to uses these modules/subroutines. In GPSR package we have developed small programs, which can be run by any person have little knowledge of computers. Following are important programs included in this package.

Program	**Purpose**
❖ fasta2sfasta	Convert fasta format to single fasta format
❖ pro2aac	To calculate amino acid composition of protein
❖ pro2aac_nt	To calculate amino acid composition of N-terminal (nt) residues of a protein
❖ pro2aac_ct	To calculate amino acid composition of C-terminal (ct) residues of a protein
❖ pro2aac_rest.pl	To calculate amino acid composition of a protein after removing N-, and C-terminal residues
❖ pro2aac_split	To calculate split amino acid composition (SSAC) of a protein
❖ pro2dpc	To calculate dipeptide composition of protein
❖ pro2dpc_nt	To calculate dipeptide composition of N-terminal (nt) residues of a protein
❖ pro2dpc_ct	To calculate dipeptide composition of C-terminal (ct) residues of a protein
❖ pro2tpc	To calculate tripeptide composition of protein
❖ add_cols	To add columns of two files
❖ col2svm	To generating SVM_light input format
❖ col_mult	To multiplying each column of input file with a number
❖ col_mult_sel	To multiplying selective columns with a number
❖ perl col_rem	To remove selective columns from a file
❖ col_ext	To extract selective columns from a file

❖ col_corr	To compute correlation co-efficient between two column
❖ col_avg	To calculate average column of two files
❖ seq2pssm_imp	To calculate PSSM matrix in column format without any normalization
❖ pssm_n1	To normalize pssm profile based on 1/(1+e-x) formula
❖ pssm_n2	To normalize pssm profile based on (numb -min)/(max -min) formula
❖ pssm_n3	To normalize pssm profile based on (numb -min)*100/(max -min) formula
❖ pssm_n4	To normalize pssm profile based on 1/(1+e-(x/100) formula
❖ pssm_comp	To compute PSSM composition (400 points)
❖ col_sig	Significance of columns in two column files
❖ pssm2pat	To generate patterns of given size from PSSM matrix
❖ pssm_smooth	To designed smooth pssm profile for plot
❖ seq2motif	To create motifs by sliding window of user defined length with option of adding terminal X
❖ motif2bin	To make binary input from the multifasta motif file
❖ blast_similarity	To perform blast

Title	Description
Fasta format	**fasta2sfasta** (Convert fasta format to single fasta format) (Pearon format) is used to represent peptide sequences or nucleic acid sequences using single-letter codes. It begins with a single-line description, followed by lines of sequence data. The description line is distinguished from the sequence data by a greater-than (">") symbol.
Single fasta format	Our programs use input sequence in single fasta format. Therefore, fasta file should first convert into single fasta format. In the single fasta format the description and sequence data merged into single line. Two hash marks (##) were present to distinguish description and sequence data.
Usage	*fasta2sfasta –i seq.fa -o seq.sfa*
-i	Input file name having sequence in fasta format
-o	Output file name that gives sequence in single fasta format
seq.fa	>seq_1 MRNRGFGRRELLVAMAMLVSVTGCARHASGARPASTTLPAGADLADRFAELERRYD ARLGVYVPATGTTAAIE >seq_2 ACGRGFGVKLACNMNNACRTYFSDVAMAMLVSVTGCARHASGARPASTTLPAGADL ADIEYRADERFAFCSTF
seq.sfa	>seq_1##MRNRGFGRRELLVAMAMLVSVTGCARHASGARPASTTLPAGADLADRFAEL ERRYDARLGVYVPATGTTAAIE >seq_2##ACGRGFGVKLACNMNNACRTYFSDVAMAMLVSVTGCARHASGARPASTTL PAGADLADIEYRADERFAFCSTF

Title	Description
	pro2aac (To calculate amino acid composition of protein) The amino acid composition in a protein is simply the percentage of the different amino acids represented in a particular protein. The aim of calculating the composition of proteins is to transform the variable length of protein sequences to fixed length feature vectors. This is an important and most crucial step during classification of proteins using machine-learning techniques because they require fixed length patterns. In addition the conversion of a protein sequence to a vector of 20 dimensions using amino acid composition will encapsulate the properties of the protein into the vector. The composition of all 20 natural amino acids were calculated by using the following equation $$\text{Composition of amino acid } i = \frac{\text{Total number of amino acid } i \text{ x } 100}{\text{Total number of all amino acids in protein}}$$ Where i can be any amino acid
Usage	*pro2aac -i seq.sfa -o seq.out*
-i	Input file name contains single fasta format
-o	Output file name gives amino acid composition
seq.sfa	>seq_1##MRNRGFGRRELLVAMAMLVSVTGCARHASGARPASTTLPAGADLADRFAEL ERRYDARLGVYVPATGTTAAIE >seq_2##ACGRGFGVKLACNMNNACRTYFSDVAMAMLVSVTGCARHASGARPASTTL PAGADLADIEYRADERFAFCSTF
seq.out	# Amino Acid Composition of proteins # A , C , D , E , F , G , H , I , K , L , M , N , P , Q , R , S , T , V , W , Y, 19.18, 1.37, 4.11, 5.48, 2.74, 9.59, 1.37, 1.37, 0.00, 9.59, 4.11, 1.37, ... 2.74, 19.18, 6.85, 5.48, 2.74, 6.85, 8.22, 1.37, 1.37, 1.37, 5.48, 4.11, 4.11, ... 2.74,
Vector	20 dimension (i.e 20 types of amino acid composition is generated)

Title	Description
	pro2aac_nt (To calculate amino acid composition of N-terminal (nt) residues of a protein) It is well known that some proteins having N-terminal signal sequence which is responsible to transport whole protein into their specific subcellular compartment like, lysosome, endoplasmic reticulum, mitochondria, and chloroplast. Evidences indicate that divergent N-terminal sequences also do influence catalytic behavior, protein-protein interactions, and intracellular distributions of enzymes. Report shows that N-terminal signal sequence can vary from 13 to 36 amino acid residues in length and having all the information needed to localize into specific location. Therefore, N-terminal information could be exploited by using amino acid composition feature to predict subcellular protein. For example: 
Usage	*pro2aac_nt -i seq.sfa -o seq.out -n 5*
-i	Input file name
-o	Output file name
-n	Number of residues to calculate composition from N-terminal
seq.sfa	>seq_1##MRNRGFGRRELLVAMAMLVSVTGCARHASGARPASTTLPAGADLADRFAELERR YDARLGVYVPATGTTAAIE >seq_2##ACGRGFGVKLACNMNNACRTYFSDVAMAMLVSVTGCARHASGARPASTTLPAGA DLADIEYRADERFAFCSTF
Seq.out	# Amino Acid Composition of 5 n-terminal residues of proteins # A , C , D , E , F , G , H , I , K , L , M , N , P , Q , R , S , T , V , W , Y, 0.00, 0.00, 0.00, 0.00, 0.00, 20.00, 0.00, 0.00, 0.00, 0.00, 20.00 ..... 0.00, 20.00, 20.00, 0.00, 0.00, 0.00, 40.00, 0.00, 0.00, 0.00, 0.00, ... 0.00,
Vector	20 dimension

Title	Description
	pro2aac_ct (To calculate amino acid composition of C-terminal (ct) residues of a protein) While the N-terminus of a protein often contains targeting signals, the C-terminus can contain retention signals for protein sorting. The most common ER retention signal is the amino acid sequence -KDEL (or -HDEL) at the C-terminus, which keeps the protein in the endoplasmic reticulum and prevents it from entering the secretory pathway. The C-terminus of proteins can be modified post-translationally, most commonly by the addition of a lipid anchor to the C-terminus that allows the protein to be inserted into a membrane without having a transmembrane domain. The c-terminal domain of RNA polymerase II typically consists of up to 52 repeats of the sequence Tyr-Ser-Pro-Thr-Ser-Pro-Ser. Other proteins often bind the C-terminal domain of RNA polymerase in order to activate polymerase activity. It is the protein domain, which is involved in the initiation of DNA transcription, the capping of the RNA transcript, and attachment to the spliceosome for RNA splicing. Therefore the information at C-terminal in could be utilized using amino acid composition feature to predict different classes of proteins. For example: 
Usage	*pro2aac_ct -i seq.sfa -o seq.out -n 5*
-i	Input file name
-o	Output file name
-n	Number of residues to calculate composition from C-terminal
seq.sfa	>seq_1##MRNRGFGRRELLVAMAMLVSVTGCARHASGARPASTTLPAGADLADRFAELERR YDARLGVYVPATGTTAAIE >seq_2##ACGRGFGVKLACNMNNACRTYFSDVAMAMLVSVTGCARHASGARPASTTLPAG ADLADIEYRADERFAFCSTF
seq.out	# Amino Acid Composition of 5 c-terminal residues of proteins # A , C , D , E , F , G , H , I , K , L , M , N , P , Q , R , S , T , V , W , Y, 40.00, 0.00, 0.00,20.00, 0.00, 0.00, 0.00,20.00, 0.00, 0.00, 0.00, 0.00, 0.00, ... 0.00, 0.00,20.00, 0.00, 0.00,40.00, 0.00, 0.00, 0.00, 0.00, 0.00, 0.00, 0.00, 0.00, ... 0.00,
Vector	20 dimension

Title	Description
	pro2aac_rest (To calculate amino acid composition of a protein after removing N-, and C-terminal residues) This program is used to calculate the composition of remaining part of a protein after removing specific residues from N-, and C-terminus. Transmembrane proteins having membrane spanning signal in the middle of protein. This program can be used to calculate the amino acid composition of middle part and successfully used in classification family of proteins. For example: 
Usage	*pro2aac_rest -i seq.sfa -o seq.out -n 5 –c 5*
-i	Input file name
-o	Output file name
-n	Number of residues removed from N-terminal
-c	Number of residues removed from C-terminal
seq.sfa	>seq_1##AAAAACCCCCGGGGG >seq_2##CCCGCAAAAASNMKL
seq.out	# Amino Acid Composition of protein after removing 5 n-terminal and 5 c-terminal residues # A , C , D , E , F , G , H , I , K , L , M , N , P , Q , R , S , T , V , W , Y, 0.00, 100.00, 0.00, 0.00, 0.00, 0.00, 0.00, 0.00, 0.00, 0.00, 0.00, ... 0.00, 100.00, 0.00, 0.00, 0.00, 0.00, 0.00, 0.00, 0.00, 0.00, 0.00, 0.00 ..... 0.00,
Vector	20 dimension

Title	Description
	pro2aac_split (To calculate split amino acid composition (SSAC) of a protein) It has been reported that some sequence motifs are present into specific region of a protein. Therefore, instead of computing the composition of whole sequence it is useful to split the sequence into different equal parts. Composition of each part is separately calculated, thus feature of region specific motifs is utilized, and added to each other. Some reports show that is increases the prediction accuracy after using this strategy. The advantage of SSAC over standard amino acid composition is that it provides greater weight to proteins that have a signal at either the N or C terminus. For Example: 
Usage	*pro2aac_split -i seq.sfa -o seq.out -n 3*
-i	Input file name
-o	Output file name
-n	Number of parts split into, here 3 i.e. three equal parts of whole protein
seq.sfa	>seq_1##AAAAACCCCCGGGGG >seq_2##CCCGCAAAAASNMKL
seq.out	# Amino Acid Composition of 3 equal parts of proteins # A , C , D , E , F , G , H , I , K , L , M , N , P , Q , R , S , T , V , W , Y, 5.00, 0.00, 5.00, 0.00, 5.00, 0.00, 0.00, 0.00, 0.00, 0.00, 0.00, 0.00, 0.00, 0.00, 0.00, 0.00, 0.00, 0.00, 0.00, 0.00, 4.00, 0.00, 0.00, 0.00, 1.00, 0.00, 0.00, 0.00, 0.00, 0.00, 0.00, 0.00, 0.00, 0.00, 0.00, 0.00, 0.00, 0.00, 0.00, 0.00, 5.00, 0.00, 1.00, 1.00, 1.00, 1.00, 0.00, 0.00, 0.00, 1.00, 0.00, 0.00, 0.00, 0.00,
Vector	60 dimension (20*3 parts)

Title	Description
	pro2dpc (To calculate dipeptide composition of protein) The dipeptide composition in a protein is simply the percentage of the different adjacent pairs of amino acids represented in a particular protein. The aim of calculating the composition of proteins is to transform the variable length of protein sequences to fixed length feature vectors. This is an important and most crucial step during classification of proteins using machine-learning techniques because they require fixed length patterns. In addition the conversion of a protein sequence to a vector of 400 dimensions using dipeptide composition will encapsulate the properties of the neighboring amino acids. A-A-A-A-A-C-C-C-C-C-G-G-G-G- N C The composition of all 400 natural amino acids were calculated by using the following equation $$\text{Composition of dipep } (i+1) = \frac{\text{Total number of amino acid } (i+1) \times 100}{\text{Total number of all possible dipeptides}}$$ Where dpep (i) is fraction or composition of dipeptide type i. Di and N are the number of dipeptide of type i and number of residues in protein i, respectively.
Usage	pro2dpc -i seq.sfa -o seq.out
-i	Input file name
-o	Output file name
seq.sfa	>seq_2##AAAAACCCCCGGGGG
seq.out	#AA , AC , AD ,….., CC ,….., CG , ….. , GG ,….., YY, 28.571, 7.143, 0.000, ….., 28.571,….., 7.143, ….., 28.571,….., 0.000
Vector	400 dimension (20*20)

Title	Description
	pro2dpc_nt (To calculate dipeptide composition of N-terminal (nt) residues of a protein) It is well known that some proteins having N-terminal signal sequence which is responsible to transport whole protein into their specific subcellular compartment like, lysosome, endoplasmic reticulum, mitochondria, and chloroplast. Evidences indicate that divergent N-terminal sequences also do influence catalytic behavior, protein-protein interactions, and intracellular distributions of enzymes. Report shows that N-terminal signal sequence can vary from 13 to 36 amino acid residues in length and having all the information needed to localize into specific location. Therefore, N-terminal information could be exploited by using dipeptide composition feature to predict subcellular protein.
Usage	*pro2dpc_nt -i seq.sfa -o seq.out -n 5*
-i	Input file name
-o	Output file name
-n	Number of residues to calculate dipeptide composition from N-terminal
seq.sfa	>seq_2##AAAAACCCCCGGGGG
Seq.out	# Dipeptide composition of 5 n-terminal residues of proteins #AA , AC , AD ,….., CC ,….., CG , ….. , GG ,….., YY, 100.00, 0.000, 0.000, ….., 00.000,….., 0.000, ….., 00.000,….., 0.000
Vector	400 dimension

Title	Description
	pro2dpc_ct (To calculate dipeptide composition of C-terminal (ct) residues of a protein) While the N-terminus of a protein often contains targeting signals, the C-terminus can contain retention signals for protein sorting. The most common ER retention signal is the amino acid sequence -KDEL (or -HDEL) at the C-terminus, which keeps the protein in the endoplasmic reticulum and prevents it from entering the secretory pathway. The C-terminus of proteins can be modified post-translationally, most commonly by the addition of a lipid anchor to the C-terminus that allows the protein to be inserted into a membrane without having a transmembrane domain. The c-terminal domain of RNA polymerase II typically consists of up to 52 repeats of the sequence Tyr-Ser-Pro-Thr-Ser-Pro-Ser. Other proteins often bind the C-terminal domain of RNA polymerase in order to activate polymerase activity. It is the protein domain, which is involved in the initiation of DNA transcription, the capping of the RNA transcript, and attachment to the spliceosome for RNA splicing. Therefore the information at C-terminal in could be utilized using dipeptide composition feature to predict different classes of proteins.
Usage	*pro2dpc_ct -i seq.sfa -o seq.out -n 5*
-i	Input file name
-o	Output file name
-n	Number of residues to calculate dipeptide composition from C-terminal
seq.sfa	>seq_2##AAAAACCCCCGGGGG
Seq.out	# Dipeptide composition of 5 n-terminal residues of proteins #AA , AC , AD ,….., CC ,….., CG , ….. , GG ,….., YY, 100.00, 0.000, 0.000, ….., 00.000,….., 0.000, ….., 100.000,….., 0.000
Vector	400 dimension

Title	Description
	pro2tpc (To calculate tripeptide composition of protein) The tripeptide composition in a protein is simply the percentage of the three adjacent amino acids represented in a particular protein. The aim of calculating the composition of proteins is to transform the variable length of protein sequences to fixed length feature vectors. This is an important and most crucial step during classification of proteins using machine-learning techniques because they require fixed length patterns. In addition the conversion of a protein sequence to a vector of 8000 dimensions using tripeptide composition will encapsulate the properties of the neighboring amino acids.  A-A-A-A-A-C-C-C-C-C-G-G-G-G- N C The composition of all 8000 natural amino acids were calculated by using the following equation $$\text{Composition of tripep (i +2)} = \frac{\text{Total number of amino acid } (i+2) \times 100}{\text{Total number of all possible tripeptides}}$$
Usage	*pro2tpc -i seq.sfa -o seq.out*
-i	Input file name
-o	Output file name
seq.sfa	>seq_2##AAAAACCCCCGGGGG
Seq.out	# Tripeptide Composition of Protein #AAA ,AAC ,AAD ,AAE ,AAF , …..,,YYW ,YYY 23.0769 , 7.6923, 0.000, 00.000, 0.000, ….., 0.000 , 0.000
Vector	8000 dimension

Title	Description
	add cols (To add columns of two files) It is used to make a hybrid method. In this two different features (e.g. amino acid composition, and dipeptided) of a sequence are added to make a more informative hybrid features.
Usage	*add_cols -i se1.out -c se2.out -o seq.out*
-i	Input file (first column file for add)
-c	Input file (second column file for add)
-o	Output file name
se1.out	Amino Acid Composition of proteins # A , C , D , E , F , G , ….., Y, 33.33,33.33, 0.00, 0.00, 0.00,33.33, ….., 0.00
se2.out	# Dipeptide Composition of Protein #AA , AC , ….., YY 28.571,7.143…..,0.00
seq.out	# Amino Acid Composition of proteins # Dinucleic Composition of Protein # A , C , D , E , F , G , …., #AA , AC, …., YY 33.33,33.33, 0.00, 0.00, 0.00, 33.33,……, 28.571,7.143…..,0.00
Vector	420 (20 for amino acid + 400 dipeptide composition)

Title	Description
	col2svm (To generating SVM_light input format) This program can convert composition output file into a format used in SVM training. In SVM format, (1) starts with +1 or –1 denotes class of sequence positive or negative respectively. (2) A numerical order is given before each value.
Usage	*col2svm -i se1.out -o svm.out -s +1*
-i	Input file name
-o	Output file name
-s	Class for svm (+1 or –1)
se1.out	Amino Acid Composition of proteins # A, C, D, E, F, G,… Y, 33.33, 33.33, 0.00, 0.00, 0.00, 33.33, ….., 0.00
svm.out	+1 1:33.330000 2:33.330000 3:0.000000 4:0.000000 5:0.000000 6:33.330000 ……. 20:0.000000
Vector	20 dimension

Title	Description
	col_mult (To multiplying each column of input file with a number) This program is used to multiply each column of input file with a specific number. This is used especially in the hybrid case to make the features equal weight. Suppose one wants to make a hybrid file of amino acid and dipeptide composition. If amino acid and dipeptide composition was added directly the values of mononucleotide is very high with respect to dinucleotide. Thus performance of SVM will be nearly similar to the performance of amino acid because the weight of dipeptide is diluted. But when we multiply the amino acid with 10 or dipeptide with 0.1 and then added to each other. There is chance that performance will increase.
Usage	*col_mult -i se1.out -o se1_mult -n 0.1*
-i	Input file name
-o	Output file name
-n	Number with which column is multiplying
se1.out	Amino Acid Composition of proteins # A , C , D , E , F , G , ….., Y, 33.33, 33.33, 0.00, 0.00, 0.00, 33.33, ….., 0.00
se1_mult	3.333000, 3.333000, 0.000000, 0.000000, 0.000000, 3.333000,….. , 0.000000,
Vector	Same as in input file

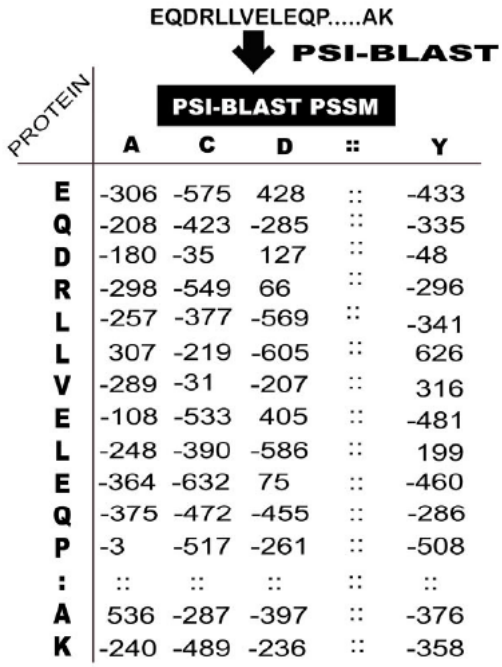
Title	Description
	col_mult_sel (To multiplying selective columns with a number) Instead of multiplying whole column, here only column from 1 to 3 are multiplied with specific number (10).
Usage	*col_mult_sel -i se1.out -o se1_mult -n 10 -a 1 -b 3*
-i	Input file name
-o	Output file name
-n	Number with which column is multiplying
-a	Number of starting column (eg 1)
-b	Number of last column (eg 3)
se1.out	Amino Acid Composition of proteins # A , C , D , E , F , G , ….., Y, 33.33, 33.33, 0.00, 0.00, 0.00, *33.33*, ….., 0.00
se1_mult	333.300000, 333.300000, 0.000000, 0.000000, 0.000000, *33.330000*,….., 0.000000
Vector	Same as in input file

Title	Description
	perl col_rem (To remove selective columns from a file) This program is used to remove specific column from files. You can remove the composition of A and C from whole file to check the importance of these amino acids in prediction methods.
Usage	*perl col_rem -i seq.out -o seq.rm -a 1 -b 2*
-i	Input file name
-o	Output file name
-a	Number of starting column (eg 1) to removed
-b	Number of last column (eg 3) removed
seq.out	# Amino Acid Composition of proteins # A , C , D , E , F , G , H , I , K , L , M , N , P , Q , R , S , T , V , W , Y, 18.60, 2.33, 4.65, 5.81, 5.81, 8.14, 1.16, 1.16, 0.00, 8.14, 3.49, 1.16, 3.49, 0.00, 13.95, 4.65, 8.14, 5.81, 0.00, 3.49,
Seq_rm	5.810000,5.810000,8.140000,1.160000,1.160000,0.000000,8.140000,3.490000, 1.160000,3.490000,0.000000,13.950000,4.650000,8.140000,5.810000,0.000000,3.490000
Vector	Total number = Total columns in the input file – total number of removed column E.g.(17=20-3)

Title	Description
	col_ext (To extract selective columns from a file) This program only takes specific column from a file. In this example we only take the feature of amino acid composition of F, G, H, I, and K as an input for SVM.
Usage	*col_ext -i seq.out -o seq.ext -a 5 -b 10*
-i	Input file name
-o	Output file name
-a	Number of starting column (eg 5) to take
-b	Number of last column (eg 10) to take
seq.out	# Amino Acid Composition of proteins # A , C , D , E , F , G , H , I , K , L , M , N , P , Q , R , S , T , V , W , Y, 18.60, 2.33, 4.65, 5.81, 5.81, 8.14, 1.16, 1.16, 0.00, 8.14, 3.49, 1.16, 3.49, 0.00, 13.95, 4.65, 8.14, 5.81, 0.00, 3.49,
Seq.ext	5.81, 8.14, 1.16, 1.16, 0.00, 8.14
Vector	Total number = Total number of column selected from input file Eg(6=from 5 to 10 colum)

Title	Description
	col_corr (To compute correlation co-efficient between two column) Correlation co-efficient indicates the strength and direction of a linear relationship between two random variables. The correlation varies between –1 to 1. The closer the coefficient is to either -1 or 1, the stronger the correlation between the variables. Value of 1 in the case of an increasing linear relationship, -1 in the case of a decreasing linear relationship, 0 in case no correlation. Example shows the correlation between amino acid A and G in the file.
Usage	*col_corr -i pos -a 1 -b 6*
-i	Input file name
-a	Number of column (eg 1)
-b	Number of column (eg 6)
pos	# Amino Acid Composition of proteins # A , C , D , E , F , G , H , I , K , L , M , N , P , Q , R , S , T , V , W , Y, 15.31, 1.30, 7.49, 3.91, 2.28, 9.45, 1.63, 3.26, 1.95, 10.10, 1.95, 2.28, 5.54, 2.28, 8.14, 3.91, 8.47, 6.84, 0.98, 2.93, 15.31, 1.30, 7.49, 3.91, 2.28, 9.45, 1.63, 3.26, 1.95, 10.10, 1.95, 2.28, 5.54, 2.28, 8.14, 3.91, 8.47, 6.84, 0.98, 2.93, 12.83, 1.60, 8.56, 2.14, 2.67, 6.95, 6.95, 2.67, 1.60, 9.09, 3.74, 3.74, 6.42, 6.42, 4.28, 5.35, 6.42, 5.35, 1.07, 2.14, 12.30, 1.60, 8.56, 2.14, 2.67, 6.95, 6.95, 2.67, 1.60, 9.09, 3.74, 3.74, 6.42, 6.42, 4.28, 5.35, 6.42, 5.88, 1.07, 2.14, 13.76, 0.53, 4.76, 4.23, 3.70, 5.29, 1.06, 3.17, 2.12, 8.47, 3.17, 3.70, 13.76, 1.59, 4.76, 9.52, 8.99, 5.82, 1.06, 0.53,
output	0.749 (a positive correlation between column 1 and 6)
Vector	Total number = Total number of column selected from input file E.g.(6=from 5 to 10 colum)

Title	Description
	col_avg (To calculate average column of two files) In this case composition value of each column of a file is added to its corresponding column of another file and means value is calculated. It can be used to generate an average feature of two different files (belonging from same family of protein) to make input in machine learning techniques. For instance, 15.31 (1st column of file pos1) + 6.87 (1st column of file pos2) = 22.18/2 = 11.09 (file out). Note: Each file should have equal number of columns and rows
Usage	*col_avg -a pos1 -b pos2 -o out*
-a	First input file name
-b	Second input file name
-o	Output file name
pos1	# Amino Acid Composition of proteins # A , C , D , E , F , G , H , I , K , L , M , N , P , Q , R , S , T , V , W , Y, 15.31, 1.30, 7.49, 3.91, 2.28, 9.45, 1.63, 3.26, 1.95,10.10, 1.95, 2.28, 5.54, 2.28, \ 8.14, 3.91, 8.47, 6.84, 0.98, 2.93, 15.31, 1.30, 7.49, 3.91, 2.28, 9.45, 1.63, 3.26, 1.95,10.10, 1.95, 2.28, 5.54, 2.28, \ 8.14, 3.91, 8.47, 6.84, 0.98, 2.93, 12.83, 1.60, 8.56, 2.14, 2.67, 6.95, 6.95, 2.67, 1.60, 9.09, 3.74, 3.74, 6.42, 6.42, \ 4.28, 5.35, 6.42, 5.35, 1.07, 2.14,
pos2	# Amino Acid Composition of proteins # A , C , D , E , F , G , H , I , K , L , M , N , P , Q , R , S , T , V , W , Y, 6.87, 1.29, 6.87, 2.15, 0.86, 6.87, 1.72, 4.72, 5.58, 9.87, 1.29, 5.15, 4.72, 5.15, 1.72,13.73, 9.01,10.30, 1.72, 0.43, 9.87, 1.29, 7.30, 3.00, 0.86, 8.58, 1.29, 4.72, 4.29, 9.87, 0.86, 3.43, 5.15, 4.29, 3.43,11.16, 7.73, 10.73, 1.72, 0.43, 12.64, 1.10, 4.40, 1.10, 2.75, 9.34, 0.55, 6.59, 3.30, 9.34, 2.20, 6.59, 6.04, 5.49, 4.40, 6.59, 7.14, 9.34, 0.55, 0.55,
out	11.09; 1.295; 7.18; 3.03; 1.57; 8.16; 1.675; 3.99; 3.765; 9.985; 1.62; 3.715; 5.13; 3.715; 4.93; 8.82; 8.74; 8.57; 1.35; 1.68; 0 12.59; 1.295; 7.395; 3.455; 1.57; 9.015; 1.46; 3.99; 3.12; 9.985; 1.405; 2.855; 5.345; 3.285; 5.785; 7.535; 8.1; 8.785; 1.35; 1.68; 0 12.735; 1.35; 6.48; 1.62; 2.71; 8.145; 3.75; 4.63; 2.45; 9.215; 2.97; 5.165; 6.23; 5.955; 4.34; 5.97; 6.78; 7.345; 0.81; 1.345; 0
Vector	Total number of column (same as in input file)

Title	Description
	seq2pssm_imp (To calculate PSSM matrix in column format without any normalization) The PSSM for each sequence was generated by performing PSI-BLAST search against specific database (e.g. nr) using different iterations (e.g. 3) with cut off e-value 0.001. For a sequence of length N residues, PSSM is represented by an NX20 matrix. Each element of this matrix, m [i, j], provides information on evolutionary conservation of residue type j at sequence position i. For example: 
Usage	*seq2pssm_imp -i seq1.fa -o pssm.out –d nr*
-i	Input file in the fasta format (not use single fasta format)
-o	Output file
-d	Database against which PSSM profile is generated
seq1.fa	>1BISA PDBID CHAIN_SEQUENC GSHMHGQVDCSPGIWQLDCTHLEGKVILVAVHVASGYIEAEVIPAETGQETAYFLLKLAG RWPVKTVHTDNGSNFTSTTVKAACEWAGIKQEFGIPYNPQSQGVIESMNKELK
pssm.out	G, 0, -300, -100, -200, -300, 599, -200, -400, -200, -400, -300, 0, -200, -200, -200, 0, -200, -300, -200, -300 S, 100, -100, 0, 0, -200, 0, -100, -200, 0, -200, -100, 100, -100, 0, -100, 400, 100, -200, -300, -200 H, -200, -300, -100, 0, -100, -200, 799, -300, -100, -300, -200, 100, -200, 0, 0, -100, -200, -300, -200, 200 M, -100, -100, -300, -200, 0, -300, -200, 100, -100, 200, 499, -200, -200, 0, -100, -100, -100, 100, -100, -100 H, -200, -300, -100, 0, -100, -200, 799, -300, -100, -300, -200, 100, -200, 0, 0, -100, -200,\-300, -200, 200

	G, 0, -300, -100, -200, -300, 599, -200, -400, -200, -400, -300, 0, -200, -200, -200, 0, -200, -300, -200, -300 Q, -100, -300, 0, 200, -300, -200, 0, -300, 100, -200, 0, 0, -100, 499, 100, 0, -100, -200, -200, -100 …….

Title	Description
	pssm_n1 (To normalize pssm profile based on 1/(1+e-x) formula) The value of PSSM matrix varies between large range which make difficult for SVM training. Thus every element of PSSM is normalized by using 1/(1+e-x) for normalization. Various formulae can be used for normalization.
Usage	*pssm_n1 -i pssm.out –o pssm_n1*
-i	Input file having pssm profile generated by using (seq2pssm_imp.pl -i seq1.fa -o pssm.out –d nr.02)
-o	Output file having normalized value
pssm.out	G, 0, -300, -100, -200, -300, 599, -200, -400, -200, -400, -300, 0, -200, -200, -200, 0, -200, -300, -200, -300 S, 100, -100, 0, 0, -200, 0, -100, -200, 0, -200, -100, 100, -100, 0, -100, 400, 100, -200, -300, -200 H, -200, -300, -100, 0, -100, -200, 799, -300, -100, -300, -200, 100, -200, 0, 0, -100, -200, -300, -200, 200 M, -100, -100, -300, -200, 0, -300, -200, 100, -100, 200, 499, -200, -200, 0, -100, -100, -100, 100, -100, -100 H, -200, -300, -100, 0, -100, -200, 799, -300, -100, -300, -200, 100, -200, 0, 0, -100, -200,-300, -200, 200 G, 0, -300, -100, -200, -300, 599, -200, -400, -200, -400, -300, 0, -200, -200, -200, 0, -200, -300, -200, -300 Q, -100, -300, 0, 200, -300, -200, 0, -300, 100, -200, 0, 0, -100, 499, 100, 0, -100, -200, -200, -100 …….
pssm_n1	G, 0.5, 5.19e-131, 3.73e-44, 1.39e-87, 5.19e-131, 1, 1.39e-87, 1.93e-174, 1.39e-87, 1.93e-174, 5.19e-131, 0.5, 1.39e-87, 1.39e-87, 1.39e-87, 0.5, 1.39e-87, 5.19e-131, 1.39e-87, 5.19e-131 S, 1, 3.73e-44, 0.5, 0.5, 1.39e-87, 0.5, 3.73e-44, 1.39e-87, 0.5, 1.39e-87, 3.73e-44, 1, 3.73e-44, 0.5, 3.73e-44, 1, 1, 1.39e-87, 5.19e-131, 1.39e-87 H, 1.39e-87, 5.19e-131, 3.73e-44, 0.5, 3.73e-44, 1.39e-87, 1, 5.19e-131, 3.73e-44, 5.19e-131, 1.39e-87, 1, 1.39e-87, 0.5, 0.5, 3.73e-44, 1.39e-87, 5.19e-131, 1.39e-87, 1 M, 3.73e-44, 3.73e-44, 5.19e-131, 1.39e-87, 0.5, 5.19e-131, 1.39e-87, 1, 3.73e-44, 1, 1, 1.39e-87, 1.39e-87, 0.5, 3.73e-44, 3.73e-44, 3.73e-44, 1, 3.73e-44, 3.73e-44 H, 1.39e-87, 5.19e-131, 3.73e-44, 0.5, 3.73e-44, 1.39e-87, 1, 5.19e-131, 3.73e-44, 5.19e-131,

1.39e-87, 1, 1.39e-87, 0.5, 0.5, 3.73e-44, 1.39e-87, 5.19e-131, 1.39e-87, 1

G, 0.5, 5.19e-131, 3.73e-44, 1.39e-87, 5.19e-131, 1, 1.39e-87, 1.93e-174, 1.39e-87, 1.93e-174, 5.19e-131, 0.5, 1.39e-87, 1.39e-87, 1.39e-87, 0.5, 1.39e-87, 5.19e-131, 1.39e-87, 5.19e-131

Q, 3.73e-44, 5.19e-131, 0.5, 1, 5.19e-131, 1.39e-87, 0.5, 5.19e-131, 1, 1.39e-87, 0.5, 0.5, 3.73e-44, 1, 1, 0.5, 3.73e-44, 1.39e-87, 1.39e-87, 3.73e-44

Title	Description
	pssm_n2 (To normalize pssm profile based on (numb -min)/(max -min) formula) The value of PSSM matrix varies between large range which make difficult for SVM training. Thus every element of PSSM is normalized by using (numb -min)/(max -min) for normalization. For example: **x-min/max-min** (Normalize PSSM in range of 0-1) **Normalized PSSM** PROTEIN A C D :: Y E 0.21 ***0.08*** 0.59 :: 0.15 Q 0.26 0.15 0.22 :: 0.20 D 0.28 0.35 0.43 :: 0.34 R 0.22 0.09 0.40 :: 0.22 L **0.24** 0.18 0.08 :: 0.19 L 0.21 0.26 0.06 :: 0.69 V 0.22 0.35 0.26 :: 0.53 E 0.31 ***0.10*** 0.57 :: 0.12 L 0.24 0.17 0.07 :: 0.47 E 0.18 ***0.05*** 0.41 :: 0.13 Q 0.18 0.13 0.14 :: 0.22 P 0.37 0.11 0.24 :: 0.11 : :: :: :: :: :: A 0.64 0.22 0.17 :: 0.18 K 0.25 0.12 0.25 :: 0.19
Usage	*pssm_n2 -i pssm.out –o pssm_n2*
-i	Input file having pssm profile generated by using (seq2pssm_imp.pl -i seq1.fa -o pssm.out –d nr.02)

-o	Output file having normalized value
pssm.out	G, 0, -300, -100, -200, -300, 599, -200, -400, -200, -400, -300, 0, -200, -200, -200, 0, -200, -300, -200, -300 S, 100, -100, 0, 0, -200, 0, -100, -200, 0, -200, -100, 100, -100, 0, -100, 400, 100, -200, -300, -200 H, -200, -300, -100, 0, -100, -200, 799, -300, -100, -300, -200, 100, -200, 0, 0, -100, -200, -300, -200, 200 M, -100, -100, -300, -200, 0, -300, -200, 100, -100, 200, 499, -200, -200, 0, -100, -100, -100, 100, -100, -100 H, -200, -300, -100, 0, -100, -200, 799, -300, -100, -300, -200, 100, -200, 0, 0, -100, -200,-300, -200, 200 G, 0, -300, -100, -200, -300, 599, -200, -400, -200, -400, -300, 0, -200, -200, -200, 0, -200, -300, -200, -300 Q, -100, -300, 0, 200, -300, -200, 0, -300, 100, -200, 0, 0, -100, 499, 100, 0, -100, -200, -200, -100 …….
pssm_n2	G, 0.26, 0.06, 0.20, 0.13, 0.06, 0.66, 0.13, 0, 0.13, 0, 0.06, 0.26, 0.13, 0.13, 0.13, 0.26, 0.13, 0.06, 0.13, 0.06 S, 0.33, 0.20, 0.26, 0.26, 0.13, 0.26, 0.20, 0.13, 0.26, 0.13, 0.20, 0.33, 0.20, 0.26, 0.20, 0.53, 0.33, 0.13, 0.06, 0.13 H, 0.13, 0.06, 0.20, 0.26, 0.20, 0.13, 0.79, 0.06, 0.20, 0.06, 0.13, 0.33, 0.13, 0.26, 0.26, 0.20, 0.13, 0.06, 0.13, 0.40 M, 0.20, 0.20, 0.06, 0.13, 0.26, 0.06, 0.13, 0.33, 0.20, 0.40, 0.59, 0.13, 0.13, 0.26, 0.20, 0.20, 0.20, 0.33, 0.20, 0.20 H, 0.13, 0.06, 0.20, 0.26, 0.20, 0.13, 0.79, 0.06, 0.20, 0.06, 0.13, 0.33, 0.13, 0.26, 0.26, 0.20, 0.13, 0.06, 0.13, 0.40 G, 0.26, 0.06, 0.20, 0.13, 0.06, 0.66, 0.13, 0, 0.13, 0, 0.06, 0.26, 0.13, 0.13, 0.13, 0.26, 0.13, 0.06, 0.13, 0.06 Q, 0.20, 0.06, 0.26, 0.40, 0.06, 0.13, 0.26, 0.06, 0.33, 0.13, 0.26, 0.26, 0.20, 0.59, 0.33, 0.26, 0.20, 0.13, 0.13, 0.2

Title	Description
	pssm_n3 (To normalize pssm profile based on (numb -min)*100/(max -min) formula) The value of PSSM matrix varies between large ranges which make difficult for SVM training. Thus every element of PSSM is normalized by using (numb -min)*100/(max -min)for normalization.
Usage	*pssm_n3 -i pssm.out –o pssm_n3*
-i	Input file having pssm profile generated by using (seq2pssm_imp.pl -i seq1.fa -o pssm.out –d nr.02)
-o	Output file having normalized value
pssm.out	G, 0, -300, -100, -200, -300, 599, -200, -400, -200, -400, -300, 0, -200, -200, -200, 0, -200, -300, -200, -300 S, 100, -100, 0, 0, -200, 0, -100, -200, 0, -200, -100, 100, -100, 0, -100, 400, 100, -200, -300, -200 H, -200, -300, -100, 0, -100, -200, 799, -300, -100, -300, -200, 100, -200, 0, 0, -100, -200, -300, -200, 200 M, -100, -100, -300, -200, 0, -300, -200, 100, -100, 200, 499, -200, -200, 0, -100, -100, -100, 100, -100, -100 H, -200, -300, -100, 0, -100, -200, 799, -300, -100, -300, -200, 100, -200, 0, 0, -100, -200,-300, -200, 200 G, 0, -300, -100, -200, -300, 599, -200, -400, -200, -400, -300, 0, -200, -200, -200, 0, -200, -300, -200, -300 Q, -100, -300, 0, 200, -300, -200, 0, -300, 100, -200, 0, 0, -100, 499, 100, 0, -100, -200, -200, -100
pssm_n3	G, 26.68, 6.67, 20.01, 13.34, 6.67, 66.64, 13.34, 0, 13.34, 0, 6.67, 26.68, 13.34, 13.34, 13.34, 26.68, 13.34, 6.67, 13.34, 6.67 S, 33.35, 20.01, 26.68, 26.68, 13.34, 26.68, 20.01, 13.34, 26.68, 13.34, 20.01, 33.35, 20.01, 26.68, 20.01, 53.36, 33.35, 13.34, 6.67, 13.34 H, 13.34, 6.67, 20.01, 26.68, 20.01, 13.34, 79.98, 6.67, 20.01, 6.67, 13.34, 33.35, 13.34, 26.68, 26.68, 20.01, 13.34, 6.67, 13.34, 40.02 M, 20.01, 20.01, 6.67, 13.34, 26.68, 6.67, 13.34, 33.35, 20.01, 40.02, 59.97, 13.34, 13.3, 26.68, 20.01, 20.01, 20.01, 33.35, 20.01, 20.01 H, 13.34, 6.67, 20.01, 26.68, 20.01, 13.34, 79.98, 6.67, 20.01, 6.67, 13.34, 33.35, 13.34, 26.68, 26.68, 20.01, 13.34, 6.67, 13.34, 40.02 G, 26.68, 6.67, 20.01, 13.34, 6.67, 66.64, 13.34, 0, 13.34, 0, 6.67, 26.68, 13.34, 13.34, 13.34, 26.68, 13.34, 6.67, 13.34, 6.67 Q, 20.01, 6.67, 26.68, 40.02, 6.67, 13.34, 26.68, 6.67, 33.35, 13.34, 26.68, 26.68, 20.01, 59.97, 33.35, 26.68, 20.01, 13.34

Title	Description
	pssm_n4 (To normalize pssm profile based on 1/(1+e-(x/100) formula) The value of PSSM matrix varies between large range which make difficult for SVM training. Thus every element of PSSM is normalized by using 1/(1+e-(x/100) for normalization.
Usage	*pssm_n4 -i pssm.out –o pssm_n4*
-i	Input file having pssm profile generated by using (seq2pssm_imp.pl -i seq1.fa -o pssm.out –d nr.02)
-o	Output file having normalized value
pssm.out	G, 0, -300, -100, -200, -300, 599, -200, -400, -200, -400, -300, 0, -200, -200, -200, 0, -200, -300, -200, -300 S, 100, -100, 0, 0, -200, 0, -100, -200, 0, -200, -100, 100, -100, 0, -100, 400, 100, -200, -300, -200 H, -200, -300, -100, 0, -100, -200, 799, -300, -100, -300, -200, 100, -200, 0, 0, -100, -200, -300, -200, 200 M, -100, -100, -300, -200, 0, -300, -200, 100, -100, 200, 499, -200, -200, 0, -100, -100, -100, 100, -100, -100 H, -200, -300, -100, 0, -100, -200, 799, -300, -100, -300, -200, 100, -200, 0, 0, -100, -200,-300, -200, 200 G, 0, -300, -100, -200, -300, 599, -200, -400, -200, -400, -300, 0, -200, -200, -200, 0, -200, -300, -200, -300 Q, -100, -300, 0, 200, -300, -200, 0, -300, 100, -200, 0, 0, -100, 499, 100, 0, -100, -200, -200, -100 …….
pssm_n4	G, 0.5, 0.04, 0.26, 0.11, 0.04, 0.99, 0.11, 0.01, 0.11, 0.01, 0.0474258731775668, 0.5, 0.11, 0.11, 0.11, 0.5, 0.11, 0.047, 0.11, 0.04 S, 0.73, 0.26, 0.5, 0.5, 0.11, 0.5, 0.26, 0.11, 0.5, 0.11, 0.26, 0.73, 0.26, 0.5, 0.26, 0.98, 0.73, 0.11, 0.04, 0.11 H, 0.11, 0.04, 0.26, 0.5, 0.26, 0.11, 0.99, 0.04, 0.26, 0.04, 0.11, 0.73, 0.119202922022118, 0.5, 0.5, 0.26, 0.11, 0.04, 0.11, 0.88 M, 0.26, 0.26, 0.04, 0.11, 0.5, 0.04, 0.11, 0.73, 0.26, 0.88, 0.99, 0.11, 0.11, 0.5, 0.26, 0.26, 0.26, 0.73, 0.26, 0.26 H, 0.11, 0.047, 0.26, 0.5, 0.26, 0.11, 0.99, 0.047, 0.26, 0.04, 0.11, 0.73, 0.11, 0.5, 0.5, 0.26, 0.11, 0.04, 0.11, 0.88 G, 0.5, 0.04, 0.26, 0.11, 0.04, 0.99, 0.11, 0.01, 0.11, 0.01, 0.04, 0.5, 0.11, 0.11, 0.11, 0.5, 0.11, 0.04, 0.11, 0.04 Q, 0.26, 0.04, 0.5, 0.88, 0.04, 0.11, 0.5, 0.04, 0.73, 0.11, 0.5, 0.5, 0.26, 0.99, 0.73, 0.5, 0.26, 0.11, 0.11, 0.26

Title	Description
	pssm_comp (To compute PSSM composition (400 points)) Here pssm matrix is coverted in a vector of dimension 400, by computing composition of occurrences of each type of amino acid corresponding to each type of amino acids in protein sequence. It means for each column we will have 20 values instead of one. Every element in this input vector was subsequently divided by the length of the sequence. The resultant matrix with 400 elements was used as input feature for SVM.
Usage	*pssm_comp -i pssm_n4 –o pssm_n4.out*
-i	Input file having pssm profile generated by using (seq2pssm_imp -i seq1.fa –o pssm.out –d nr.02) and then scaled by using (pssm_n4.pl -i pssm.out –o pssm_n4)
-o	Output file having 400 elements
pssm_n4	G, 0.5, 0.04, 0.26, 0.11, 0.04, 0.99, 0.11, 0.01, 0.11, 0.01, 0.04, 0.5, 0.11, 0.11, 0.11, 0.5, 0.11, 0.04, 0.11, 0.04 S, 0.73, 0.26, 0.5, 0.5, 0.11, 0.5, 0.26, 0.11, 0.5, 0.11, 0.26, 0.73, 0.26, 0.5, 0.26, 0.98, 0.73, 0.11, 0.04, 0.11 H, 0.11, 0.04, 0.26, 0.5, 0.26, 0.11, 0.99, 0.047, 0.26, 0.047, 0.11, 0.73, 0.11, 0.5, 0.5, 0.26, 0.11, 0.04, 0.11, 0.88 M, 0.26, 0.26, 0.04, 0.11, 0.5, 0.04, 0.11, 0.73, 0.26, 0.88, 0.99, 0.11, 0.11, 0.5, 0.26, 0.26, 0.26, 0.73, 0.26, 0.26 H, 0.11, 0.04, 0.26, 0.5, 0.26, 0.11, 0.99, 0.04, 0.26, 0.04, 0.11, 0.73, 0.11, 0.5, 0.5, 0.26, 0.11, 0.04, 0.11, 0.88 G, 0.5, 0.04, 0.26, 0.11, 0.04, 0.99, 0.11, 0.01, 0.11, 0.01, 0.04, 0.5, 0.11, 0.11, 0.11, 0.5, 0.11, 0.04, 0.11, 0.04 Q, 0.26, 0.04, 0.5, 0.88, 0.04, 0.11, 0.5, 0.04, 0.73, 0.11, 0.5, 0.5, 0.26, 0.99, 0.73, 0.5, 0.26, 0.11, 0.11, 0.26
pssm_n4.out	0.98, 0.50, 0.11, 0.26, 0.11, 0.50, 0.11, 0.26, 0.26, 0.26, 0.26, 0.11, 0.26, 0.26894142, 0.26, 0.73, 0.50, 0.50, 0.04, 0.11, 0.50, 0.99, 0.04, 0.01, 0.11, 0.04, 0.04, 0.26, 0.04, 0.26, 0.26, 0.04, 0.04, 0.04, 0.04, 0.26, 0.26, 0.26, 0.11, 0.11, ……..
Vector	400

Title	Description
	col_sig (significance of columns in two column files) This program used to calculate significant of each column in two different file. If any one want to compare the positive and negative file of amino acid composition. Like Differences in positive-negative, its significance, average of each colomn in positive and each column in negative, standard deviation. Output result will give comparison of each column.
Usage	*col_sig -i file1 -j file2 >out*
-i	Input file1 of positive example
-j	Input file2 of negative example
file1	# Amino Acid Composition of proteins # A , C , D , E , F , G , H , I , K , L , M , N , P , Q , R , S , T , V , W , Y 7.88,1.27,4.05,6.39,3.62,7.88,2.13,6.61,5.75,10.23,3.62,2.98,3.41,5.11,5.54,6.39,4.47,7.03,1.70,3.83 5.46,1.12,7.14,6.72,3.78,5.46,1.68,4.76,6.58,10.64,0.98,7.42,2.52,4.06,4.90,9.38,8.54,5.04,0.98,2.80 8.96,2.06,4.82,7.58,4.13,6.20,0.69,4.82,7.58,13.10,1.37,2.75,4.82,8.96,6.89,4.13,2.75,6.20,0.00,2.64
file2	# Amino Acid Composition of proteins # A , C , D , E , F , G , H , I , K , L , M , N , P , Q , R , S , T , V , W , Y 8.55,0.65,3.94,9.86,2.63,8.55,0.00,3.28,11.84,13.81,2.63,3.28,1.31,3.94,3.94,6.57,1.31,8.55,0.65,1.89 13.29,2.53,3.16,2.53,5.69,14.55,1.89,5.69,3.16,6.32,3.16,3.16,6.96,2.53,6.32,6.32,2.53,6.32,3.16,2.18 9.88,0.48,7.45,8.91,5.18,3.56,0.48,4.70,11.02,9.88,2.10,6.48,3.89,4.21,3.24,5.99,5.02,2.91,0.16,4.87
out	# Parameters Measured: % Difference, Significance, Average1, Average2, Standard Deviation(SD1), SD2 Column 1: -34.83, -490.31, 7.43, 10.57, 0.88, 0.39 Column 2: 19.44, 69.33, 1.48, 1.22, 0.33, 0.42 Column 3: 9.51, 53.98, 5.34, 4.85, 0.29, 1.50 Column 4: -2.89, -28.15, 6.90, 7.10, 0.39, 1.04 Column 5: -15.71, -234.15, 3.84, 4.50, 0.16, 0.39 Column 6: -30.79, -145.77, 6.51, 8.89, 0.18, 3.07 Column 7: 61.49, 218.36, 1.50, 0.79, 0.46, 0.17 Column 8: 16.83, 408.83, 5.4, 4.56, 0.33, 0.07 Column 9: -26.55, -214.22, 6.64, 8.67, 0.54, 1.35 Column 10: 12.34, 240.14, 11.32, 10.01, 1.02, 0.07 Column 11: -27.64, -193.90, 1.99, 2.63, 0.35, 0.30 Column 12: 1.76, 6.98, 4.38, 4.31, 0.94, 1.25 Column 13: -12.27, -115.47, 3.58, 4.05, 0.71, 0.09 Column 14: 51.68, 241.21, 6.04, 3.56, 1.68, 0.37

	Column 15: 24.79, 185.57, 5.78, 4.50, 0.64, 0.73
	Column 16: 5.22, 41.72, 6.63, 6.30, 1.44, 0.17
	Column 17: 56.04, 174.63, 5.26, 2.95, 1.44, 1.19
	Column 18: 2.69, 17.94, 6.09, 5.93, 0.06, 1.74
	Column 19: -38.94, -72.75, 0.89, 1.32, 0.516, 0.67
	Column 20: 3.47, 15.63, 3.093, 2.98, 0.26, 1.09

Title	Description
	pssm2pat (To generate patterns of given size from PSSM matrix) Here we generate PSSM matrix from different window size. If we want to generate 5-window the 100 vector long matrix representing H (middle) of nucleotide. For starting nucleotide zero
Usage	*pssm2pat -i pssm.out –o pssm_pat –w 5*
-i	Input file having pssm profile generated by using (seq2pssm_imp.pl -i seq1.fa -o pssm.out –d
-o	Output file
-w	Window size generated from PSSM matrix
pssm.out	G, 0, -300, -100, -200, -300, 599, -200, -400, -200, -400, -300, 0, -200, -200, -200, 0, -200, -300, -200, -300 S, 100, -100, 0, 0, -200, 0, -100, -200, 0, -200, -100, 100, -100, 0, -100, 400, 100, -200, -300, -200 H, -200, -300, -100, 0, -100, -200, 799, -300, -100, -300, -200, 100, -200, 0, 0, -100, -200, -300, -200, 200 M, -100, -100, -300, -200, 0, -300, -200, 100, -100, 200, 499, -200, -200, 0, -100, -100, -100, 100, -100, -100 H, -200, -300, -100, 0, -100, -200, 799, -300, -100, -300, -200, 100, -200, 0, 0, -100, -200, -300, -200, 200 G, 0, -300, -100, -200, -300, 599, -200, -400, -200, -400, -300, 0, -200, -200, -200, 0, -200, -300, -200, -300 Q, -100, -300, 0, 200, -300, -200, 0, -300, 100, -200, 0, 0, -100, 499, 100, 0, -100, -200, -200, -100
pssm_pat	# Pattern Window size 5 generated from PSSM matrix. Each line represents pattern for central residue 0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,-300,-100,-200,-300,599,-200,-400,-2 0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,-300,-100,-200,-300,599,-200,-400,-200,-400,-300,0,-200,-200,-200,0,-200 100,200,499,-200,-200,0,-100,-100,-100,100,-100,-100 ……
Vector	20*window size (20*5=100)

Title	Description
	pssm_smooth (To designed smooth pssm profile for plot) Here we generate smooth matrix from different window size. If we want to generate 5-window matrix, take two nucleotide matrix forms upstream and downstream and add all five values from each column and divided by five to make average. The matrix of each nucleotide is 20. Each matrix represents about it matrix neighbour nucleotide. Therefore a smooth graph will be generated.
Usage	*pssm_smooth -i pssm.out –o pssm_pat –w 5*
-i	Input file having pssm profile generated by using (seq2pssm_imp.pl -i seq1.fa -o pssm.out –d nr.02)
-o	Output file
-w	Window size generated from PSSM matrix
pssm.out	G, 0, -300, -100, -200, -300, 599, -200, -400, -200, -400, -300, 0, -200, -200, -200, 0, -200, -300, -200, -300 S, 100, -100, 0, 0, -200, 0, -100, -200, 0, -200, -100, 100, -100, 0, -100, 400, 100, -200, -300, -200 H, -200, -300, -100, 0, -100, -200, 799, -300, -100, -300, -200, 100, -200, 0, 0, -100, -200, -300, -200, 200 M, -100, -100, -300, -200, 0, -300, -200, 100, -100, 200, 499, -200, -200, 0, -100, -100, -100, 100, -100, -100 H, -200, -300, -100, 0, -100, -200, 799, -300, -100, -300, -200, 100, -200, 0, 0, -100, -200, -300, -200, 200 G, 0, -300, -100, -200, -300, 599, -200, -400, -200, -400, -300, 0, -200, -200, -200, 0, -200, -300, -200, -300 Q, -100, -300, 0, 200, -300, -200, 0, -300, 100, -200, 0, 0, -100, 499, 100, 0, -100, -200, -200, -100
smooth.out	G, -40, -340, -160, -200, -300, 379.2, -60.2, -400, -200, -380, -200.2, 0, -260, -160, -200, 40, -200, -320, -280, -260 S, -80, -340, -160, -160, -260, 219.4, 139.6, -380, -180, -360, -180.2, 20, -260, -120, -160, 20, -200, -320, -280, -160 H, -80, -340, -160, -160, -260, 219.4, 139.6, -380, -180, -360, -180.2, 20, -260, -120, -160, 20, -200, -320, -280, -160 M, -100, -340, -140, -80, -260, 59.6, 179.6, -360, -120, -320, -120.2, 20, -240, 19.8, -100, 20, -180, -300, -280, -120 H, -120, -340, -120, 0, -260, -100.2, 219.6, -340, -60, -280, -60.2, 20, -220, 159.6, -40, 20, -160, -280, -280, -80 G, -160, -380, -120, 40, -280, -140.2, 239.6, -360, -40, -280, -40.2, 0, -220, 259.4, 0, -60, -200, -280, -260, -60 Q, -140, -380, -100, 80, -320, -140.2, 79.8, -360, 0, -260, -0.2, -20, -200, 359.2, 20, -40, -180, -260, -260, -120
Vector	20

Title	Description
	seq2motif (To create motifs by sliding window of user defined length) This program creates motif of defined length. Additional 'X' at the end of sequence is added to make complete pattern. The binary pattern is generated using the motifs
Usage	*seq2motif -i seq1.fa -o motif.out -w 5*
-i	Input file in single fasta format
-o	Output file
-w	Window size to create a pattern
seq1.fa	>seq_1##GSHMHGQVDCSPGIWQLDCTHLEGK
motif_1.out	>seq_1 XXGSH XGSHM GSHMH SHMHG HMHGQ MHGQV HGQVD GQVDC QVDCS VDCSP DCSPG CSPGI SPGIW PGIWQ GIWQL IWQLD WQLDC QLDCT LDCTH DCTHL CTHLE THLEG HLEGK LEGKX EGKXX

Title	Description
	seq2motif _simple (To create motifs by sliding window of user defined length) This program creates motif of defined length. This program is similar to seq2motif program but generates motifs without adding 'X' at the end. Binary pattern is generated using these motifs.
Usage	*seq2motif_simple -i seq1.fa -o motif_2.out -w 5*
-i	Input file in single fasta format
-o	Output file
-w	Window size to create a pattern
seq1.fa	>seq_1##GSHMHGQVDCSPGIWQLDCTHLEGK
motif_2.out	>seq_1 GSHMH SHMHG HMHGQ MHGQV HGQVD GQVDC QVDCS VDCSP DCSPG CSPGI SPGIW PGIWQ GIWQL IWQLD WQLDC QLDCT LDCTH DCTHL CTHLE THLEG HLEGK

Title	Description
	motif2bin (To make binary input from the multifasta motif file) It generates binary input from the multifasta motif file of fixed length into column format.
Usage	*motif2bin -i motif_1.out -o bin.out -x y*
-i	Input in multifasta file (seq2motif.pl -i seq1.fa -o motif.out -w 5)
-o	Output file
-x	If additional X is added in pattern then y (for yes), or n (for no)
motif_1.out	>seq_1 XXGSH XGSHM GSHMH SHMHG HMHGQ MHGQV HGQVD GQVDC QVDCS VDCSP DCSPG CSPGI SPGIW PGIWQ GIWQL IWQLD WQLDC QLDCT LDCTH DCTHL CTHLE THLEG HLEGK LEGKX EGKXX
bin.out	0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 1, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 1, 0, 0, 0, 0, 0, 1, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 1, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 1, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 1, 0, 0, 0, 0, 0, 1, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0,0, 0, 0, 0, 0,0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 1, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 1, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 1, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0 ……
Vector	20*window size (20*5=100)

Title	Description
	blast similarity (To perform blast) Basic Local Alignment Search Tool, or BLAST, is an algorithm for comparing primary biological sequence information, such as the amino-acid sequences of different proteins or the nucleotides of DNA sequences. A BLAST search enables a researcher to compare a query sequence with a library or database of sequences, and identify library sequences that resemble the query sequence above a certain threshold. For example, following the discovery of a previously unknown protein in the mouse, a scientist will typically perform a BLAST search of the protein database (nr) to see if humans carry a similar protein; BLAST will identify sequences in the previously known database that resemble the mouse protein based on similarity of sequence.
Usage	*blast_similarity -i fasta -d nr -j 3 -e 1 –o blast.out*
-i	Input file of Fasta format
-o	Output result
-d	Database used blast
-j	Number of Iteration
-e	Cut off Evalue
fasta	>amla_1 ASDATAYAACVAYANMANNNAMAKLAWQAPTCAGYAAKTGCVQRATRQOPKALVNAA SDREW >amla_2 ACDEFGHIKLMNPQRSTVWMRNRGFGRRELLVAMAMLVSVTGCARHASGARPASTTLPA GADLADRFAELERRYDARLG
blast.out	>amla_1 Zero Zero >amla_2 ref|NP_216584.1| blaC [Mycobacterium tuberculosis H37Rv] >gi|158... 2e-27

Stand-alone Programs

Installation of GPSR 1.0 package locally

- **GPSR 1.0:** This package developed mainly for UNIX machines. It can be downloaded from www.imtech.res.in/raghava/gpsr/

- **Prerequisite softwares for GPSR 1.0 package**

  SVM^{light}: freely available software (academic). It can be downloaded (old: version 5.0 and new 6.1) from http://download.joachims.org/svm_light/current/ svm_light.tar.gz.

  HMMER: freely available software (academic). User can download the source code from ftp://ftp.genetics.wustl.edu/pub/eddy/hmmer/

  MEME/MAST: freely available software (academic). User can download the source code from http://meme.nbcr.net/meme4_1/meme-download.html

  BLAST: freely available software (academic). User can download the source code from http://www.ncbi.nlm.nih.gov/BLAST/download.shtml

  cdk.jar: freely available software (academic). User can download the source code from http://sourceforge.net/projects/cdk/

- **To uncompress gpsr.tar.gz, execute the following command**
  $ tar -zxf gpsr.tar.gz

- **To install package**
  $ cd gpsr
  $ perl install.pl

  During installation it will ask for the path of required softwares like Perl, BLAST, SVM old (version 5.0), SVM New (version 6.1), MEME/MAST etc. The entire program will be installed in the bin folder.

- **To update package**
  $ perl update.pl
  Once you have installed the gpsr package and wish to update the package or

change/add the path of any software later, you may run update.pl instead of install.pl.

ESLPred2

Application:
ESLpred2 trained using organism specific and generalized datasets can be used for the prediction of eukaryotic subcellular localizations. The webserver is available at http://www.imtech.res.in/raghava/eslpred2/

Introduction:
In this post genomic era, functional annotation and characterization of nearly millions of raw protein sequences, erupted by incredible sequencing projects, are some of the inescapable challenges that has been baffling the scientific community in order to bridge the mounting gap between number of unknown and annotated proteins. This crisis entails the development of computational methods that would help in predicting functions of proteins expeditiously as well as economically. One of the fundamental and popular indirect strategies for assigning function is the identification of subcellular compartments of proteins as knowledge about localization can provide important indications about protein functions. After PSORT the first method developed to predict the subcellular localizations, ample of novice, improved, generalized and organism specific prediction methods have been developed for predicting subcellular locations of eukaryotic and prokaryotic proteins, namely, NNPSL, PSORTB, FKNN, TargetP, SubLoc, SignalP, CELLO, LOCnet, PSLpred, HSLPred, PLOC, Mutiloc, Proteome Analyst, LOCtree, TSSub, BaCelLo and Esub8 using different datasets and protein input features. In 2004, our group has combined the information of similarity search with sequence composition based attributes (ESLPred) and achieved accuracy up to 88%. In ESLPred2, a systematic approach has been taken to improve the prediction quality of eukaryotic subcellular localizations using PSI-BLAST generated PSSM profiles along with compositional attributes and similarity search based information for the training of SVM. The present method has achieved a highest success rate for the prediction of localizations with good overall and average accuracy, and hence, compliments the existing subcellular localization prediction methods.

Datasets:
ESLPred2 was trained using the latest dataset, which was earlier used for developing BaCelLo method. The dataset was retrieved from SWISSPROT version 48.0 and divided into three subsets on the basis of kingdoms- animal with 2597 sequences; fungi with 1198 sequences and 491 sequences were from plant. The major attraction of this dataset was the stringent cut-off value of 30% used to reduce the similarity between sequences. The

first two datasets covered 4 major localizations such as cytoplasm, mitochondria, nuclear, and extracellular, whereas, plant dataset included chloroplast class along with four major localizations. In addition, RH2427 dataset was also used to train a generalized model for prediction of eukaryotic proteins subcellular localizations.

Results

The hybrid approach based module which incorporated similarity search based information with amino acid composition of a single sequence (whole and N-terminal) and profiles for RH2427 dataset attained an overall accuracy to ~94% and average accuracy for four localizations to 93.1%. Using this hybrid approach, cytoplasmic, mitochondrial, nuclear, and extracellular proteins has been predicted with 89.6%, 90.7%, 96.4%, and 95.7% of accuracies respectively. Additionally, ESLpred2 has also been able to attain best accuracies of 80.8,75.9%, and 76.6% for kingdom specific animal, fungi and plant proteins respectively, which is the best accuracy reported till date for the same dataset. Hence, ESLpred2 provides more crucial and promising features for prediction of eukaryotic subcellular localizations coupled with kingdom specific prediction SVM models. An interesting feature of the present method is the hybrid of different protein features, such as composition of PSSM profile, whole and N-terminal composition of sequence and similarity search based results, which supported the assignment of the subcellular localization of proteins more reliably and with high accuracy irrespective of redundancy in the training datasets. The present method is able to complement all existing subcellular location prediction methods.

Usage of standalone version

perl eslpred2 -i <seq_file> -m <method> -k <organism> -o <output_file>

- **Seq_file** is a file containing protein sequences (single or multiple) in fasta format.
- **Method** defines the 3 modules for the prediction of subcellular localizations such as
  a) Amino acid compositions (1);
  b) PSSM (2)
  c) Hybrid module for AAC, PSSM and PSI-BLAST based similarity (3).
- **Organism** defines the models based on training dataset such as
  a) A for Animal dataset;
  b) F for Fungi dataset;
  c) P for Plant dataset.
  d) G for generalized dataset (RH2427)
- **Output_file** defines the name of output file for storing results
-

Publication:

Garg A and Raghava GPS (2008) ESLpred2: Improved method for predicting subcellular localization of eukaryotic proteins. **BMC Bioinformatics**, 9:503.

ESLPred

Application
ESLPred is a SVM-based method for the prediction of subcellular localization of eukaryotic proteins. The webserver is available at http://www.imtech.res.in/raghava/eslpred/

Introduction
Large-scale genome sequencing projects make interpretation of genomic sequence data increasingly important, so does the need to functionally annotate this data. The determination of subcellular localization of a protein can provide important clues to elucidate the function of the protein. Therefore, prediction of subcellular localization of proteins is an important step in understanding the biochemical function of proteins. In the past, various methods have been developed to predict the subcellular location of proteins using different approaches. The similarity search in which a sequence is searched against an experimentally annotated database, is a technique commonly used to assign function to a protein, including its subcellular location. This approach fails in the absence of significant similarity between query and target protein sequences. Another way to predict subcellular localization of proteins is to identify sequence motifs such as signal peptide or nuclear localization signal. The major limitation of motif-based methods is that all proteins residing in a compartment do not have universal motifs.
To overcome these limitations, in the past numerous studies have been carried out to predict subcellular localization based on the features of protein sequence. The subcellular localization prediction methods are based either on recognition of N-terminal sorting signals or on the composition of amino acids. In ESLPred, a systematic attempt has been made to achieve higher prediction accuracy for subcellular localization of eukaryotic proteins from their different features. The SVM modules were developed based on the following features of a protein: (i) amino acid composition (commonly used in the literature for classification of proteins), (ii) overall physico-chemical properties (e.g. hydrophobicity, hydrophilicity, polarity) and (iii) dipeptide compositions (e.g. ala–ala, ala–leu, val–ser). In addition, a similarity search based module, EuPSI-BLAST, was also constructed using PSI-BLAST to predict the localization of a protein. Finally, a hybrid SVM module was developed using all three features of proteins mentioned above and prediction results of EuPSI-BLAST.

Datasets
The dataset used in devloping ESLPred was also used in the development of SubLoc and NNPSL. This dataset was generated from version 33.0 of SWISS-PROT by Reinhardt and Hubbard (RH2427). The dataset consisted of complete and non-redundant proteins

with less than 90% sequence identity whose subcellular localization is experimentally determined. This dataset consisted of a total of 2427 eukaryotic proteins (1097 nuclear, 684 cytoplasmic, 321 mitochondrial and 325 extracellular proteins).

Results

Support vector machine (SVM) has been used to predict the subcellular location of eukaryotic proteins from their different features such as amino acid composition, dipeptide composition and physico-chemical properties. The SVM module based on dipeptide composition performed better than the SVM modules based on amino acid composition or physico-chemical properties. In addition, PSI-BLAST was also used to search the query sequence against the dataset of proteins (experimentally annotated proteins) to predict its subcellular location. In order to improve the prediction accuracy, we developed a hybrid module using all features of a protein, which consisted of an input vector of 458 dimensions (400 dipeptide compositions, 33 properties, 20 amino acid compositions of the protein and 5 from PSI-BLAST output). Using this hybrid approach, the prediction accuracies of nuclear, cytoplasmic, mitochondrial and extracellular proteins reached 95.3, 85.2, 68.2 and 88.9%, respectively. The overall prediction accuracy of SVM modules based on amino acid composition, physico-chemical properties, dipeptide composition and the hybrid approach was 78.1, 77.8, 82.9 and 88.0%, respectively. The accuracy of all the modules was evaluated using a 5-fold cross-validation technique. Assigning a reliability index (reliability index ≥3), 73.5% of prediction can be made with an accuracy of 96.4%.

Usage of standalone version

perl eslpred -i <seq_file> -m <method> -o <output_file>

- **Seq_file** is a file containing protein sequences (single or multiple) in fasta format.
- **Method** defines the 5 trained SVM modules for the prediction of subcellular localizations such as
  a) Amino acid compositions (1);
  b) Overall physico-chemical properties (2);
  c) Dipeptide compositions (3);
  d) PSI-BLAST similarity based (4);
  e) Hybrid module (5).
- **Output_file** defines the name of output file for storing results

Publication

Bhasin M and Raghava GPS (2004) ESLpred: SVM Based Method for Subcellular Localization of Eukaryotic Proteins using Dipeptide Composition and PSI-BLAST. Nucleic Acids Research 32:W414-9.

HSLPred

Application
HSLPred is a SVM-based method for the prediction of subcellular localizations of human proteins. The webserver is available at http://www.imtech.res.in/raghava/hslpred/

Introduction
The successful completion of a human genome project has yielded huge amount of sequence data. Analysis of this data to extract the biological information can have profound implications on biomedical research. Therefore, mining of biological information or functional annotation of piled up sequence data is a major challenge to the modern scientific community. Determination of functions of all of these proteins using experimental approaches is a difficult and time-consuming task. Traditionally, the similarity search-based tool has been used for functional annotations of proteins. This approach fails when unknown query protein does not have significant homology to proteins of known functions. The functions of the proteins are closely related to its cellular attributes, such as subcellular localization and its association with the lipid bilayer (subcellular localization) hence, the related proteins must be localized in the same cellular compartment to cooperate toward a common function. In addition, information on the localization of proteins with known function may provide insight about its involvement in specific metabolic pathways. Therefore, an attempt has been made to predict subcellular localization of proteins to elucidate the function. Several methods have been devised earlier to predict the subcellular localization of the eukaryotic and prokaryotic proteins using different approaches and data sets. To the best of our knowledge, there is no method for the prediction of subcellular localization of human proteins. Availability of sequence data of human genes in recent years demands a reliable and accurate method for prediction of subcellular localization of human proteins.
HSLpred is based on different features of the proteins such as amino acid and dipeptide composition of proteins. In addition, a similarity search-based module, HuPSI-BLAST, has also been developed, using PSI-BLAST to predict the localization of human proteins. Further, SVM module "hybrid1" has been developed using amino acid composition, traditional dipeptide composition, and results of PSI-BLAST prediction. The SVM modules based on higher order dipeptide compositions (i + 2, i + 3, and i + 4) and combinations of various feature-based modules have also been constructed. In addition, the performance of HSLPred has also been assessed on various mammalian and nonmammalian genomes and on an independent data set. It was observed that this method can predict the subcellular localization of human proteins and proteins from related genomes with high accuracy. In other words, our method can also be used for the prediction of subcellular localization of mammalian proteins.

Datasets

The dataset of human proteins used to devlop HSLpred was extracted from special release of SWISSPROT database. Final non-redundant data set consisted of a total of 3532 human proteins (840 cytoplasmic, 315 mitochondrial, 858 nuclear, 1519 plasma membrane). The dataset is available at www.imtech.res.in/raghava/hslpred.

Results

SVM based modules for predicting subcellular localization using traditional amino acid and dipeptide (i + 1) composition achieved overall accuracy of 76.6 and 77.8%, respectively. PSI-BLAST, when carried out using a similarity-based search against a nonredundant data base of experimentally annotated proteins, yielded 73.3% accuracy. To gain further insight, a hybrid module (hybrid1) was developed based on amino acid composition, dipeptide composition, and similarity information and attained better accuracy of 84.9%. In addition, SVM modules based on a different higher order dipeptide i.e. i + 2, i + 3, and i + 4 were also constructed for the prediction of subcellular localization of human proteins, and overall accuracy of 79.7, 77.5, and 77.1% was accomplished, respectively. Furthermore, another SVM module hybrid2 was developed using traditional dipeptide (i + 1) and higher order dipeptide (i + 2, i + 3, and i + 4) compositions, which gave an overall accuracy of 81.3%. We also developed SVM module hybrid3 (final) based on amino acid composition, traditional and higher order dipeptide compositions, and PSI-BLAST output and achieved an overall accuracy of 84.4%.

Usage of standalone version

perl hslpred -i <seq_file> -m <method> -o <output_file>

- **Seq_file** is a file containing protein sequences (single or multiple) in fasta format.
- **Method** defines the 4 trained SVM modules for the prediction of subcellular localizations such as
  a) Amino acid compositions (1);
  b) Dipeptide compositions (2);
  c) PSI-BLAST similarity based (3);
  d) Hybrid module (a+b+c+d) (4).
- **Output_file** defines the name of output file for storing results

Publication

Garg A, Bhasin M and Raghava GP (2005) SVM-based method for subcellular localization of human proteins using amino acid compositions, their order and similarity search. J Biol Chem 280:14427-32.

PSLpred

Application

PSLpred is a SVM-based method for the prediction of subcellular localizations of prokaryotic proteins. The webserver is available at http://www.imtech.res.in/raghva/pslpred/

Introduction

Prokaryotes are the causative agent of most of the deadly disease and widespread of epidemics, hence, biologists are paying much attention for the functional annotation of prokaryotic proteins. This may further guide the determination of virulence factors as well as new pattern of resistance for antiobiotic agents in pathogenic bacteria. Hence, prediction of protein subcellular localization (an alternative to functional annotation) of gram-negative bacteria would be very useful in the field of molecular biology, cell biology, pharmacology, and medical science. A number of methods such as PSORT I, PSORT-B and NNPSL have been developed for predicting subcellular localization of bacterial proteins based on different datasets and computational techniques. The accuracies reported by these methods vary between 60 and 81%. Recently, a support vector machines (SVM) based method, CELLO trained using n-peptide compositions has been developed for predicting subcellular localization of bacterial proteins. This method has achieved an overall accuracy of 89% that is better than existing methods for subcellular localization
of prokaryotic proteins. Despite the overall improved performance, CELLO predicts extracellular proteins with a fair accuracy of 78.9%, proteins that may represent important virulence factors in pathogenic microorganisms. PSLpred a SVM based method has been devloped for the prediction of subcellular localization of prokaryotic proteins using input features such as amino acid and dipeptide composition, physico-chemical properties along with similarity search based results.

Datasets

The data set used in the present study is the same as that used for developing the methods CELLO and PSORT-B, respectively. This data set has been generatedfrom SWISSPROT release 40.29 and consisted of a total of 1443 proteins belonging to different subcellular localizations. We have excluded 141 proteins residing in more than one subcellular locations and used the remaining 1302 proteins (248 cytoplasmic, 268 inner membrane, 244 periplasmic, 352 outer membrane and 190 extracellular) for the development of PSLpred.

Results

PSLpred is a hybrid approach-based method that integrates PSI-BLAST and three SVM modules based on compositions of residues, dipeptides and physico-chemical properties and predicts the subcellular localization of gram-negative bacterial proteins with an overall accuracy of 91.2%. The prediction accuracies of 90.7, 86.8, 90.3, 95.2 and 90.6% were attained for cytoplasmic, extracellular, inner-membrane, outer-membrane and periplasmic proteins, respectively. Furthermore, PSLpred was able to predict 74% of sequences with an average prediction accuracy of 98% at RI = 5. The performance of the hybrid module was compared with methods such as CELLO, PSORT-B, which were also developed from the same data set. It has been observed that overall performance of the hybrid module is nearly 2% higher than CELLO and 16% higher than that of PSORT-B. Hence PSLpred is more accurate for the subcellular localization of prokaryotic proteins.

Usage of standalone version

perl pslpred -i <seq_file> -m <method> -o <output_file>

- **Seq_file** is a file containing protein sequences (single or multiple) in fasta format.
- **Method** defines the 5 trained SVM modules for the prediction of subcellular localizations such as
  a) Amino acid compositions (1);
  b) Physico-chemical properties (2);
  c) Dipeptide compositions (3);
  d) PSI-BLAST similarity based (4);
  e) Hybrid module (5).
- **Output_file** defines the name of output file for storing results

Publication

Bhasin M. Garg A and Raghava GPS (2005) PSLpred: prediction of subcellular localization of bacterial proteins. Bioinformatics 21(10):2522-4.

SRTPred

Application

SRTPred is a SVM-based method for the classification of protein sequence as secretory or non-secretory protein. The webserver is available at http://www.imtech.res.in/raghava/srtpred/

Introduction

Protein secretion is a universal process which occurs in all organisms and has tremendous importance to biological research. In case of pathogenic microorganisms, secretory pathways deliver virulence factors to their sites of action, soluble extracellular enzymes into the surrounding medium, or for specifically targeting proteins to the host cell. In several instances, protein secretion pathways are similar to those involved in assembly of bacterial appendages. Further, several secretory proteins has been identified as a major target protein for the development of drugs. Hence, development of automatic method for the prediction of secretory proteins would be a help for the studies aim towards deciphering secretory pathways and also lead to the identification of novel drug targets with greater value for biomedical research.

Until now, many methods have been developed for the classification and prediction of subcellular localizations of proteins based on signal peptide (SPs), mainly SignalP and pTarget. TargetP is a neural-network based method that discriminates between proteins destined for the mitochondrion, the chloroplast, the secretory pathways and other localizations with a success rate of 85.3% (overall) and sensitivity of 0.96 for non-plant secretory proteins. Whereas, neural network based method, SignalP (version 3.0) method has been able to achieve high sensitivity of 0.99 and overall accuracy of 0.93 for eukaryotic signal peptide discrimination. Though achieving higher prediction accuracy for classical secreted proteins, these methods, unfortunately fail during the prediction of proteins without SP. Hence, non-classical secreted proteins also demand automated method for the prediction. Recently, a webserver SecretomeP has been developed to predict non-classical secreted proteins, based on an idea that extracellular proteins share certain features regardless of the pathway used to secrete them. It is a neural network based method that has used several features of protein such as number of atoms, positively charged residues, propeptide cleavage site, protein sorting, low complexity regions, and transmembrane helices as an input to train network. Despite considering large number of protein features, the method has achieved a false positive prediction that is less than 5% at a low sensitivity value of 40%. Till date, there is not any method available that can predict secretory proteins, irrespective of pathways/SPs, with better accuracy. SRTPred is an automated method that can predict secretory proteins (irrespective of N-terminal SP) based on different features of whole protein sequence.

Dataset

The data set used in the present study, consisted of 6975 mammalian protein sequences. Out of which 3321 sequences were extracellular proteins secreted via classical and non-classical pathways (positive examples), whereas the remaining 3654 proteins were annotated as cytoplasmic and/or the nuclear (negative examples). Previously, the same dataset was used to develop a method SecretomeP and available publicly at http,//www.cbs.dtu.dk/services/SecretomeP-1.0/datasets.php. The sequences were extracted from Swiss-Prot database on the basis of subcellular localization annotations in the comment block.

Results

SRTpred is a systematic attempt to predict secretory proteins irrespective of presence or absence of N-terminal signal peptides (also known as classical and non-classical secreted proteins respectively), using machine-learning techniques; artificial neural network (ANN) and support vector machine (SVM). We trained and tested our methods on a dataset of 3321 secretory and 3654 non-secretory mammalian proteins using five-fold cross-validation technique. First, ANN-based modules have been developed for predicting secretory proteins using 33 physico-chemical properties, amino acid composition and dipeptide composition and achieved accuracies of 73.1%, 76.1% and 77.1%, respectively. Similarly, SVM-based modules using 33 physico-chemical properties, amino acid, and dipeptide composition have been able to achieve accuracies 77.4%, 79.4% and 79.9%, respectively. In addition, BLAST and PSI-BLAST modules designed for predicting secretory proteins based on similarity search achieved 23.4% and 26.9% accuracy, respectively. Finally, we developed a hybrid-approach by integrating amino acid and dipeptide composition based SVM modules and PSI-BLAST module that increased the accuracy to 83.2%, which is significantly better than individual modules. We also achieved high sensitivity of 60.4% with low value of 5% false positive predictions using hybrid module.

Usage of standalone version

perl srtpred -i <seq_file> -m <method> -t <threshold value) -o <output_file>

- **Seq_file** is a file containing protein sequences (single or multiple) in fasta format.
- **Method** defines the 5 trained SVM modules for the prediction of secretory proteins such as
  a) Amino acid compositions (1);
  b) Properties based (2);
  c) Dipeptide compositions (3);
  d) PSI-BLAST similarity based (4);
  e) Hybrid module of a+c+d (5).

- **Threshold_value** defines the selection of threshold value in the range of -1.5 to 1.5
- **Output_file** defines the name of output file for storing results

Publication

Garg A and Raghava GPS (2008) A machine learning based method for the prediction of secretory proteins using amino acid composition, their order and similarity-search. **In Silico Biology** 8:129-40.

OxyPred

Application

OxyPred is a SVM based method to predict the Oxygen Binding Proteins such as Erythrocruorin, Hemoglobin, Myoglobin, Hemerithrin, Leghemoglobin and Hemocyanin. The webserver is available at http://www.imtech.res.in/raghava/oxypred/

Introduction

Oxygen-binding proteins are widely present in eukaryotes ranging from non-vertebrates to humans. Moreover, these proteins have also been reported to be present in many prokaryotes and protozoans. The occurrence of oxygen-binding proteins in all kingdoms of organisms, though not in all organisms, shows their biological importance. Extensive studies on oxygen-binding proteins have categorized them into six different broad types, including ery-throcruorin, hemerythrin, hemocyanin, hemoglobin, leghemoglobin, and myoglobin, each has its own functional characteristics and structure with unique oxygen-binding capacity. These oxygen-binding proteins are crucial for the survival of any living organism. With the advancement in sequencing technology, the size of protein sequence databases is growing at an exponential rate. Thus it is much needed to develop bioinformatics methods for functional annotation of proteins, particularly for identifying oxygen-binding proteins. We have developed a reliable SVM-based method for predicting and classifying oxygen-binding proteins using different residue compositions.

Dataset

The sequences of oxygen-binding proteins and non-oxygen-binding proteins from the Swiss-Prot database (http://www.expasy.org/sprot/). In order to obtain a high-quality dataset, we removed all those proteins annotated as “fragments", “isoforms", “potentials", “similarity", or “probables" and created a non-redundant dataset where no two proteins have a similarity more than 90% using PROSET software. Our final dataset consisted of 672 oxygen-binding proteins and 700 non-oxygen binding proteins. These 672 oxygen-binding proteins were then classified into six different classes, consisting of 20 erythrocruorin, 31 hemerythrin, 77 hemocyanin, 486 hemoglobin, 13 heghemoglobin, and 45 myoglobin proteins.

Results

SVM modules were developed using amino acid composition and dipeptide composition for predicting oxygen-binding proteins and achieved maximum accuracy of 85.5% and 87.8%, respectively. Secondly, SVM module was developed based on amino acid composition, classifying the predicted oxygen-binding proteins into six classes with accuracy of 95.8%, 97.5%, 97.5%, 96.9%, 99.4%, and 96.0% for erythrocruorin,

hemerythrin, hemocyanin, hemoglobin, leghemoglobin, and myoglobin proteins, respectively. Finally, a module was developed using dipeptide composition for classifying the oxygen-binding proteins, and achieved maximum accuracy of 96.1%, 98.7%, 98.7%,
85.6%, 99.6%, and 93.3% for the above six classes, respectively.

Usage of standalone version

perl oxypred -i <seq_file> -m <method> -o <output_file>

- **Seq_file** is a file containing protein sequences (single or multiple) in fasta format.
- **Method** defines the 2 trained SVM modules for the prediction such as
  a) Amino acid compositions (1);
  b) Dipeptide compositions (2);
- **Output_file** defines the name of output file for storing results

Publication

Muthukrishnan S, Garg A and Raghava GPS (2007) OxyPred: Prediction and Classification of Oxygen-Binding Proteins. **Genomics, Proteomics & Bioinformatics** 5:250-2

DPROT

Application

DPROT is a SVM based method to predict disordered proteins using evolutionary information. The webserver is available at http://www.imtech.res.in/raghava/dprot/

Introduction

The knowledge of three dimensional (3D) structure of a protein is essential to deduce its biological function. Since, prediction of secondary structure is an intermediate step in structure determination, hence, in the past number of secondary and super secondary structure prediction methods have been developed by our. However, past few years have seen a growing interest in structural studies of proteins, focusing comprehensively on the study of proteins which are structurally disordered, often, known as disordered proteins. These proteins have been gaining high attention from biologists, since their involvement in various physiological disorders which could be protein deposition diseases such as Alzheimer's and Parkinson's diseases became evident. From the structure point of view, a disordered protein, or disordered regions, are those lacking a specific tertiary structure and is composed of an ensemble of conformations, usually with distinct and dynamic _ and _. These proteins in their purified state at neutral pH, either have been shown experimentally or are predicted to lack ordered structure. Existence of disorder is determined by overall protein dynamics rather than by local secondary structure. These proteins are also referred as “natively unfolded” or “intrinsically unstructured”.

Several predictors have been developed in the past for instance PONDR (Jones et al. 2003), DISOPRED2 (Ward et al. 2004), GlobPlot (Linding et al. 2003), DISEMBL (Linding et al. 2003), FoldIndex (Sussman et al. 2005) and RONN (Yang et al. 2005) etc. for predicting disorder proteins/regions. All these predictors exploit various attributes of the protein sequence such as amino acid compositions, flexibility, charge, hydropaths, PSIBLAST profiles, propensities for secondary structure and random coils etc. On the other hand, IUPRED (Dosztanyi et al. 2005) which is based on inter-residue interactions, predicts regions that lack a well defined 3D structure under native conditions, whilst, FoldUnfold (Galzitskaya et al. 2006), predicts disordered regions by estimating the number of contacts of the whole protein. Recently, a predictor POODLE has been developed (Shimizu et al. 2007), which can predict disordered proteins with a high sensitivity value of 72.3% and an accuracy of 97.7%. POODLE is based on joachims’ spectral graph transducer (SGT), which is a binary classification based on semi-supervised learning. Despite gaining such higher prediction accuracy, the method seems to be insensitive for the set partially disordered proteins. This insensitivity might be due to the utilization of single protein feature namely amino acid composition for prediction.

The present study has been undertaken to further improve the prediction performance for

classifying ordered and disordered proteins with an introduction to new input feature like secondary structure composition, along with conventionally used protein features such as amino acid composition, dipeptide composition, and Position Specific Scoring Matrices (PSSM) composition. However, best performance was observed for PSSM based module capturing the multiple sequence alignment information for the prediction of disordered proteins, hence, the module has been implemented on web server and standalone version.

Dataset

A representative dataset consisting of 608 proteins: 526 ordered and 82 disordered proteins. The same dataset was earlier used to develop the POODLE web server. Its raw dataset retrieved from Disprot (version 3.3), was later on processed by following an intensive protocol. Additionally, a data set of 417 partially disordered proteins was also used for independent testing.

Results

The association of structurally disordered proteins with a number of diseases has engendered an enormous interest and hence, demands a prediction method which would comprehend their study at molecular level expeditiously. DPROT is computational method for prediction of disordered proteins using sequence and profile compositions as input features for the training of SVM models. First, we developed the amino acid and dipeptide composition based SVM modules, which were able to yield sensitivity of 75.6 and 73.2% along with MCC values of 0.75 and 0.60 respectively. In addition, the use of predicted secondary structure content (coil, sheet and helices) in the form of composition values attained 76.8% and 0.77 of sensitivity and MCC values. Finally, training of SVM models using evolutionary information hidden in multiple sequence alignment profile improved the prediction performance by achieving sensitivity value of 78% and MCC of 0.78. Furthermore, the same SVM module when evaluated on an independent dataset of partially disordered proteins provided 86.6% of correct predictions.

Usage of standalone version

perl dprot -i <seq_file> -t <threshold value) -o <output_file>

- **Seq_file** is a file containing protein sequences (single or multiple) in fasta format.
- **Threshold_value** defines the selection of threshold value in the rangeof -1.5 to 1.5
- **Output_file** defines the name of output file for storing results

Publication

Sethi D, Garg A and Raghava GPS (2008) DPROT: Prediction of Disordered Proteins using Evolutionary Information. **Amino Acids** 35:599-605.

NRpred

Application

NRpred is a SVM based tool for the classification of nuclear receptors on the basis of amino acid composition or dipeptide composition**.** The webserver is available at http://www.imtech.res.in/raghava/nrpred/

Introduction

The recognition of nuclear receptors is crucial because many of them are potential drug targets for developing therapeutic strategies for diseases like breast cancer and diabetes. Nuclear receptors are one of the most abundant classes of transcriptional regulators, which regulate diverse functions during reproduction, metabolism and development. Nuclear receptors function as ligand activated transcriptional factors, providing a direct link between signaling molecules that control these processes and transcriptional responses. Besides this, nuclear receptors share a common structural organization. All nuclear receptors consist of six distinct regions or domians: N- and C- terminal highly variable regions (A/B & F domains) that contain one or more transactivation regions, a central well conserved DNA binding domain (C), a non conserved hinge region (D) that contains Nuclear Localization Signal (NLS) and a moderately conserved ligand binding domain (E) (4). The DNA binding domain (C region) of nuclear receptors consists of two zinc fingers, which act as a signature for this superfamily. The presence of these zinc fingers facilitate the recognition of nuclear receptors from genome sequence using simple similarity based search tools like BLAST and FASTA . On the other hand, the major limitation of these search tools is that they are not able to classify the subfamilies of nuclear receptors. The nuclear receptors have been classified to seven subfamilies, which include thyroid and estrogen hormone like receptor according to nucleaRDB database. However, classification of these subfamilies is difficult by using the phylogeny or BLAST based tools due to scarcity of data for some subfamilies. Thus, there is a crucial need for methods to enable automated assignment of nuclear receptor subfamilies. In this report, we have made an attempt to develop a method for recognizing the subfamilies of nuclear receptors. We are able to design a method for recognizing the four subfamilies of nuclear receptors: Thyroid hormone like (TR,RAR, ROR), HNF4-like (HNF4, RXR, TLL, Coup, USP), Estrogen like (ER, ERR, GR, MR, PR,
AR) and Fushi tarazu-F1 like (SFI, FTF, FTZ-F1). Sequences for the other three subfamilies are not available in significant number (less than 10). The classification of nuclear receptors to various subfamilies was done on the basis of amino acid composition and dipeptide composition. The amino acid and dipeptide composition are simplistic approaches to produce patterns of fixed length from the protein sequences of varying length. In the past, the amino acid composition has been used to predict the domains

structural class and subcellular localization of proteins. The dipeptide composition is also widely used to encapsulate the global information and giving a fixed pattern length of 400. In the past, dipeptide composition has been used for the prediction of subcellular localization of proteins and for fold recognition. In this study, Support Vector Machines (SVM) was applied to classify nuclear receptors.

Dataset

The data for four subfamilies of nuclear receptors was obtained from nucleaRDB database available at http://www.receptors.org/NR/. All the entries, which were not marked as fragments, were extracted from the database by text parsing method. The initial dataset had 577 sequences belonging to four subfamilies of nuclear receptors. Redundancy was reduced so that no sequence had >=90% sequence identity with any other sequence in the data set, using PROSET software. The final dataset contains 282 sequences belonging to different subfamilies of nuclear receptors.

Results

The performance of all classifiers was evaluated using 5-fold cross validation test. It was found that different subfamilies of nuclear receptors were quite closely correlated in terms of amino acid composition as well as dipeptide composition. The overall accuracy of amino acid composition and dipeptide composition based classifiers were 82.6% and 97.5%, respectively. Therefore, our results proven that different subfamilies of nuclear receptors are predictable with considerable accuracy using amino acid or dipeptide composition.

Usage of standalone version

perl nrpred -i <seq_file> -m <method> -o <output_file>

- **Seq_file** is a file containing protein sequences (single or multiple) in fasta format.
- **Method** defines the 2 trained SVM modules for the prediction such as
  a) Amino acid compositions (1);
  b) Dipeptide compositions (2);
- **Output_file** defines the name of output file for storing results

Publication

Bhasin M and Raghava GPS (2004) Classification of nuclear receptors based on amino acid composition and dipeptide composition. **J Biol Chem** 279:23262-6

PLPred

Application

PLPred is a SVM based method to predict and classify plastids. The webserver is available at http://www.imtech.res.in/raghava/plpred/

Introduction

Plastids are characteristic plant cell organelles that perform essential biosynthetic and metabolic functions. These include photosynthetic carbon fixation, and the synthesis of amino acids, fatty acids, starch and secondary metabolites such as pigments. On the basis of their structure, pigment composition (colour), metabolism and function, plastids are classified as chloroplasts in photosynthetically active tissues, chromoplasts in fruits and petals, amyloplasts in roots, etioplasts in dark-grown seedlings and elaioplasts that are found in the seed endosperm. Although plastids are of significant biological interest, our current understanding of the metabolite functions and capacities of different plastid types is still limited. However, Proteomics is a powerful approach to map the complete set of plastid proteins and to infer plastid-type specific metabolite functions, only a few proteomic approaches have been reported. Besides time consuming, the experimental approaches face several other constraints; for example, the chloroplast proteome analysis is nearing saturation because the detection of new proteins is constrained by highly abundant photosynthetic proteins that dominate the proteome of photosynthetically active chloroplasts. To circumvent these constraints and to increase proteome coverage, the development of highly efficient computational prediction tools is another complementary approach to provide useful global information about the possible evolution of the plastid proteome. PLpred is an attempt in this direction which is a Support Vector Machine (SVM) based two-phase prediction tool for identifying as well as classifying the plastid proteins.

Various features of a protein sequence viz. Amino acid composition, Dipeptide composition and Split Amino Acid Composition (SAAC) were exploited in the development of this prediction method. Secondly, the similarity search-based PSI-BLAST module was also developed. In addition, N-terminal and C-terminal amino acid composition based SVM modules as well as the Hybrid-based classifiers were also developed in order to encapsulate more comprehensive information from a protein sequence. Conclusively, the best modules were selected and made available on this server for classification of plastid proteins.

Dataset

To infer various plastid-type specific functions, only a few proteomic approaches have been reported and thus, very less experimentally proved plastid protein sequences are available in the public databases. Protein sequences for Etioplast and Chloroplast were

downloaded from PLprot database. For Amyloplast and Chromoplast sequences, whole of the 'UniProt' was searched for the available sequences. A total of 1033 protein sequences were extracted from these two databases for the said four plastid-types. For generating data for phase-I training process, all the above 1033 plastid-type protein sequences were combined to form one 'positive dataset' for developing various phase-I prediction classifiers. For generating 'negative dataset', we downloaded some experimentally annotated sequences belonging to cytoplasm and nucleus cellular localizations. As both the cytoplasmic and nucleus targeted proteins lack signal peptides in their N-terminus region as compared to the plastid proteins which always consist of N-terminal targeting peptides, these sequences were considered as better option for creating 'negative dataset'. Hence, the 'negative dataset' for training in phase-I consisted of 103 cytoplasmic sequences from rice, 226 cytoplasmic sequences from arabidopsis and 704 nuclear proteins from arabidopsis (Total = 1033 sequences).

Results

The present prediction tool is a two-phase process and was developed in two stages. In the first stage, when a user submits a query sequence, it is firstly predicted as plastid or non-plastid protein (phase-I). If the query protein is predicted as 'Plastid' through phase-I after that it will be passed to the next stage, which is the classification stage (phase-II). Here, the query protein will be classified to one of its plastid-type (Chloroplast, Chromoplast, Etioplast or Amyloplast) class. For developing hybrid module, we combined the traditional amino acid composition technique and the dipetide composition with the four-parts based amino acid composition along with the similarity-based Psi-Blast approach. Thus, the SVM input vector pattern in this case was 505 (20 for amino acid, 400 for dipeptide, 80 for four-parts based amino acid composition and 5 for Psi-Blast output as binary representation). Best results were again obtained with the RBF kernel with an overall accuracy of 90.13% and an overall MCC of 0.76.

Usage of standalone version

perl plpred -i <seq_file> -m <method> -t <threshold value) -o <output_file>

- **Seq_file** is a file containing protein sequences (single or multiple) in fasta format.
- **Method** defines the 5 trained SVM modules for the prediction of plastids such as
  a) Amino acid compositions (1);
  b) Dipeptide compositions (2);
  c) Split four parts compositions (3);
  d) PSI-BLAST similarity based (4);
  e) Hybrid module of a+b+c+d (5).
- **Threshold_value** defines the selection of threshold value in the range of -1.5 to 1.5

- **Output_file** defines the name of output file for storing results

AntiBP

Application:

AntiBP is a server that predicts whether a peptide possesses antibacterial properties or not. The web server can be accesses through http://www.imtech.res.in/raghava/antibp.

Introduction

Antibacterial peptides are important components of the innate immune system, used by the host to protect itself from different types of pathogenic bacteria. Over the last few decades, the search for new drugs and drug targets has prompted an interest in these antibacterial peptides. We analyzed 486 antibacterial peptides, obtained from antimicrobial peptide database APD, in order to understand the preference of amino acid residues at specific positions in these peptides. It was observed that certain types of residues are preferred over others in antibacterial peptides, particularly at the N and C terminus. These observations encouraged us to develop a method for predicting antibacterial peptides in proteins from their amino acid sequence.

Results

First, the N-terminal residues were used for predicting antibacterial peptides using Artificial Neural Network (ANN), Quantitative Matrices (QM) and Support Vector Machine (SVM), which resulted in an accuracy of 83.63%, 84.78% and 87.85%, respectively. Then, the C-terminal residues were used for developing prediction methods, which resulted in an accuracy of 77.34%, 82.03% and 85.16% using ANN, QM and SVM, respectively. Finally, ANN, QM and SVM models were developed using N and C terminal residues, which achieved an accuracy of 88.17%, 90.37% and 92.11%, respectively. All the models developed in this study were evaluated using five-fold cross validation technique. These models were also tested on an independent or blind dataset.

Usage of standalone version

antibp -i <seq_file> -t <terminus> -a <approach> -t <threshold> -o <output_file>

-i inputFile (in Fasta format)
-t Terminus to be used for pediction (N or C or NC).
-a Approach used for prediction (SVM or ANN or QM).
-t [optional] Threshold for Prediction [default value is 0 for SVM approach, 0.6 for ANN and -0.2 for QM based approach]
-o Output Result file

Reference

Sneh Lata, B K Sharma, G P S Raghava. Analysis and prediction of antibacterial peptides. BMC Bioinformatics 2007, 8:263

PolyApred

Application

PolyApred is a support vector machine (SVM) based method for the prediction of polyadenylation signal (PAS) in human DNA sequence. The webserver is available at http://www.imtech.res.in/raghava/polyapred/

Introduction:

Polyadenylation signal plays key role in determining the site for addition of polyadenylated tail to nascent mRNA and its mutation(s) are reported in many diseases. Identification of poly (A) sites is important to determine the gene boundary like, the last exon and 3′ UTR, which plays critical role in mRNA stability and localization. In the past, a number of methods have been developed for predicting poly(A) signals in a given nucleotide sequence by exploiting nucleotide feature around PAS signals.

In this method we utilized the features of region specific nucleotide frequency around the PAS signals and achieved highest accuracy.

Dataset

The investigations were performed on two different dataset: (a) Positive dataset containing 2327 sequences and each sequence is 206 nt long having poly (A) signal at the centre (101 to 106 nt). (b) Negative dataset containing 2333 sequences and each sequence is 206 nt long extracted from coding region of gene that have AATAAA at the centre (101 to 106 nt).

Results

In this study, Support Vector Machine (SVM) models have been developed for predicting poly(A) signals in a DNA sequence using 100 nucleotides, each upstream and downstream of this signal. Here, we introduced a novel split nucleotide frequency technique, and the models, thus, developed achieved maximum Matthews correlation coefficient (MCC) of 0.58, 0.69, 0.70 and 0.69 using mononucleotide, dinucleotide, trinucleotide, and tetranucleotide frequencies, respectively. Finally, a hybrid model developed using combination of dinucleotide, 2nd order dinucleotide and tetranucleotide frequencies, and achieved maximum MCC of 0.72. Moreover, for independent datasets this model achieved a precision ranging from 75.8 - 95.7% with a sensitivity of 57%, which is better than any other known methods.

Usage of standalone version

perl polyapred -i inputFile -t threshold -o Output_Result_File

-i inputFile (in Fasta format)
-t Threshold for SVM based [default = 0]
-o Output Result file

PolyApred program: In this query sequence (in Fasta format) leads to the following path

1. bin/fasta2sfasta: present in the bin directory of the package to make multi-fasta format sequence into simple-fasta (SFASTA) format
2. bin/mot_polya: Take 100 nt upstream and 100 nt downstream of a putative PAS signal (six nt). Each 100 nt long sequence is divided into two equal region (50 nt).
3. bin/ freq_polya: calculate nucleotide frequency of each region and make input for SVM.
4. SVM classify with model svm_models/polyapred/model_polya

Publication

Ahmed F, Kumar M, Raghava GPS. (2009) Prediction of polyadenylation signals in human DNA sequences using nucleotide frequencies. In Silico Biology. 9:007.

ABCpred

Application
The aim of ABCpred server is to predict B cell epitope(s) in an antigen sequence, using artificial neural network. The webserver is available at
http://www.imtech.res.in/raghava/abcpred/

Introduction
B-cell epitopes play a vital role in the development of peptide vaccines, in diagnosis of diseases, and also for allergy research. Experimental methods used for characterizing epitopes are time consuming and demand large resources. The availability of epitope prediction method(s) can rapidly aid experimenters in simplifying this problem. The standard feed-forward (FNN) and recurrent neural network (RNN) have been used in this study for predicting B-cell epitopes in an antigenic sequence.

Dataset
B-cell epitopes were obtained from B cell epitope database (BCIPEP), which contains 2479 continuous epitopes, including 654 immunodominant, 1617 immunogenic epitopes. All the identical epitopes and non-immunogenic peptides were removed; finally we got 700 unique experimentally proved continuous B cell epitopes. The dataset covers a wide range of pathogenic group like virus, bacteria, protozoa and fungi. Final dataset consists of 700 B-cell epitopes and 700 non-epitopes or random peptides (equal length and same frequency generated from SWISS-PROT).
Result
The server is able to predict epitopes with 65.93% accuracy using recurrent neural network.Users can select window length of 10, 12, 14, 16 and 20 as predicted epitope length. It presents the results in tabular frame, which will provide sequence name, pattern, prediction score and its position.

Usage of standalone version
perl polyapred -i inputFile -t threshold –w 16 -o Output_Result_File

-i inputFile (in Fasta format)
-t Threshold [0.1 to 1, default = 0.5]
-w Window length [10, 12, 14, 16, 18, or 20]
-o Output Result file

ABCpred program: In this query sequence (in Fasta format) leads to the following path

1. bin/fasta2sfasta: present in the bin directory of the package to make multi-fasta format sequence into simple-fasta (SFASTA) format
2. bin/ seq2motif_simple: create motifs by sliding window of defined length
3. bin/ motif2_binsnns.pl: make binary input for ANN from the motif file
4. ANN classify with model svm_models/abcpred/neural10- neural 20

Publication:

Saha, S and Raghava G.P.S. (2006) Prediction of Continuous B-cell Epitopes in an Antigen Using Recurrent Neural Network. Proteins,65(1),40-48.

WebCdk

Application:

In the process of drug development and QSAR model building there is need to calculate various descriptors of a molecule. These descriptors are used as a feature vector. There are many free as well as paid softwares which are generally used for descriptor calculation. Presented program is a java based standalone version of WebCdk web server which uses the cdk descriptor calculation tool, making it more user friendly, faster and with support of commonly used molecular file formats. Program can calculate geometrical, electrical, topological and constitutional descriptors for a given molecule and can handle many molecules at a time.

Running webCdk locally:

Program to Calculate descriptors (Topological, Electrical, Geometrical and Constitutional) for smile, mol and sdf format file.

README.txt	README file enclose in the gpsr/src/webcdk
test.smi, test.mol, test.sdf	few test files
1) /gpsr/src/webcdk/runWebCdk	this is main program to run
2) /gpsr/src/webcdk/packageWebCdk.class	JAVA program, required by main perl program and CLASSPATH should be defined for this
3) /gpsr/src/webcdk/packageWebCdk.java	Source code for 'packageWebCdk' program
4) /gpsr/src/webcdk/cdk-1.0.3.jar	cdk jar file on which WebCdk is based

Important Instructions for running this program

For running this program user need to have JAVA and CDK installed in the system and set the path for JAVA accordingly.
User can check the path of java by typing 'env' from the command prompt.
In the 'PATH' field java is set e.g. /usr/java/jdk1.6.0_06/bin:/usr/java/jdk1.6.0_06/jre/bin
Set the path by adding in the .bashrc or .bash_profile file

or for the bash shell user type 'export PATH=$PATH:/usr/jdk/jdk1.5.0_06/bin/:/usr/jdk/jdk1.5.0_06/jre1.6.0_06/bin/:/usr/jdk/jdk1.5.0_06/jre/bin/' (JAVA Directory)

set the webcdk directory in ur path as export PATH=$PATH:/gpsr/src/webcdk/

Setting CLASSPATH for CDK

1) The CDK should be in the 'CLASSPATH' field.

user can check by 'env' or add in the the CLASSPATH field of .bashrc or .bash_profile file.

or for the bash shell user type 'export CLASSPATH=$CLASSPATH:/home/user/gpsr/src/webcdk/cdk-1.0.3.jar' (CDK Directory)

2) Add the webcdk installation directory in the CLASSPATH as mentioned above

export CLASSPATH=$CLASSPATH:/home/user/gpsr/src/webcdk/

eg. For the bash shell user type 'export CLASSPATH=$CLASSPATH:/home/user/gpsr/src/webcdk/packageWebCdk.class' (webcdk package Directory)

Without setting path for JAVA user will probably get the error like 'Exception in thread "main" java.lang.NoClassDefFoundError: packageWebCdk'

Usage:

perl runWebCdk -i inputFile -f fileFormat -d descriptor -o resultFile

-i InputFile in smile, mol or sdf format

-f [smile|mol|sdf] Input file format; 'smile' for smile, 'mol' for mol and 'sdf' for sdf file format

-d [a|t|e|g|c] Descriptor required to calculate; 'a' for total 178 descriptors, 't' for topological, 'e' for electrical, 'g' for geometrical and 'c' for constitutional descriptors respectively

-o OutPut result file

Reference:

http://crdd.osdd.net:8081/webcdk/

NADbinder

Application:

The program predicts the NAD (Nicotinamide adenine dinucleotide) binding residues in a protein. NAD along with other small molecular cofactors like FAD, ATP etc play a very important role in the regulation of enzyme activity. We hope that presented tool will aid in the advancement of ligand-protein interaction studies.

Introduction:

In the post-genomic era understanding protein-ligand interaction is very promising because many proteins recruit small molecular ligands or co-factors such as ATP, NAD and FAD etc. for their function. The first step for understanding protein–ligand interaction would be to analyze binding of these ligands to the specific amino acid residues. In the present study we have developed 2 modules for the prediction of NAD binding residue. One approach is using binary feature and second is using evolutionary information coupled with SVM (support vector machine) to classify an amino acid residue as interacting or non-interacting. We got initial dataset of NAD interacting proteins from PDB (Protein Data Bank) by using LPC (Ligand protein contact) tool. We had 1545 amino acid sequences reported to bind to NAD but with redundancy. After reducing the redundancy at a cutoff of 40% we were left with just 195 sequences. With the help of Binary features we were able to achieve an accuracy of 74% while evolutionary information in the form of PSSM (Position specific scoring matrix) gave an accuracy of 85%.

Program description:
Usage:
perl nadbinder -i inputFile -m method -t threshold -o Output_Result_File
-i inputFile (in Fasta format)
-m [b|p] [optional] 'b' for Binary method 'p' for PSSM based prediction [default=p]
-t [optional] Threshold for SVM based Prediction [default =-0.2]
-o Output Result file

Supporting programs needed: (for each program detail description see the package documentation)

There are two approaches used in the program

1. **Binary approach -**

Here binary feature is used as a input for SVM ie sequences are converted into 1 and 0 matrix of 21xwindow length vector size, where each residue comes at the center of the motif.

The steps are as follows-

1. bin/fasta2sfasta -i seq_temp -o seq_temp.sfasta (sequence is converted to SFASTA format)
2. bin/seq2motif -i seq_temp.sfasta -w 17 -x y -o temp.mot (SFASTA seq to motifs with X aa)
5. bin/motif2bin -i temp.mot -x y -o temp.bin (Motif to Binary conversion ie 0 and 1 format)
6. bin/col2svm -i temp.bin -o temp.svm -s 0 (column format)
7. svm classify with svm_models/NADbinder/model_binary_nadbinder model file
8. bin/count_pred_binary_center.pl -p temp.score -s seq_temp.sfasta -o result -t thresold (for comparing the predicted score and threshold for each center residue).

2. **PSSM based approach -**

Here query sequence is converted into PSSM matrix, parsed, normalized, converted into patterns according to window size in such a manner that each center residue's matrix value is spanned by adjacent residues value, making vector size 20x window length for SVM input.

1. bin/seq2pssm_imp -i seq_temp -o temp.pssm -d swissprot (query sequence is converted into PSSM matrix by using blastpgp and makemat programs)
2. bin/pssm2pat -i temp.pssm -w 17 -o temp.pat (PSSM to patterns of 17 window size)
3. bin/col2svm -i temp.pat -o temp.svm -s 0 (column to SVM readable format, 20x17 vector)
4. svm classify with svm_models/NADbinder/model_pssm_nadbinder model file
5. bin/count_pred_binary_center.pl -p temp.score -s seq_temp.sfasta -o result -t thresold (for comparing the predicted score and threshold for each center residue).

Sample input:

>1AF3_B|PDBID_CHAIN_SEQUENCE
MSQSNRELVVDFLSYKLSQKGYSWSQFSDVEENRTEAPEETEPERETPSAINGNP
SWHLADSPAVNGATGHSSSLDAREVIPMAAVKQALREAGDEFELRYRRAFSDLT
SQLHITPGTAYQSFEQVVNELFRDGVNWGRIVAFFSFGGALCVESVDKEMQVLV

SRIASWMATYLNDHLEPWIQENGGWDTFVDLYG

Sample output:

lowercase: non-interacting residues ; UPPERCASE followed by '*' : INTERACTING RESIDUES
>1AF3_B_PDBID_CHAIN_SEQUENC Length = 196
msqS*nR*E*lV*vdF*lsyklS*qkG*Y*sW*S*qfS*dveenrteaP*eetepereT*psainG*npswh
L*adsP*aV*ngatG*hsssldarevipmaavK*qalR*eaG*D*eF*elryrrafsD*L*tsqlhitpG*taY*
Q*sF*eqV*vnE*lF*R*D*G*V*nwG*rI*V*aF*F*sF*gG*A*lcV*esvdK*emqvL*vsR*ia
swM*A*T*Y*lnD*hlE*pwiqE*N*G*gW*D*tF*V*dL*yg

Detail Residue wise View

Pos	Residue	Score	Prediction
1	m	-0.58103577	non-interacting
2	s	-0.47046973	non-interacting
3	q	-0.46166293	non-interacting
4	S*	0.29743143	INTERACTING
5	n	-0.29965193	non-interacting
6	R*	-0.18128689	INTERACTING
7	E*	0.26258585	INTERACTING
8	l	-0.41980352	non-interacting
9	V*	-0.18798209	INTERACTING
10	v	-0.30472188	non-interacting
11	d	-0.74795141	non-interacting
12	F*	0.15331554	INTERACTING
13	l	-1.3255221	non-interacting
14	s	-0.43654671	non-interacting
15	y	-0.30118048	non-interacting
16	k	-1.0193331	non-interacting
17	l	-1.0320422	non-interacting
18	S*	0.3288692	INTERACTING
19	q	-0.70314709	non-interacting
20	k	-0.60352651	non-interacting
21	G*	0.46728328	INTERACTING
22	Y*	0.76776858	INTERACTING

Reference:

www.imtech.res.in/raghava/NADbinder

MITPRED

Application:

The program is able to classify any query protein into Mitochondrial or Non-mitochondrial localization. Stand-alone version is very useful for running whole proteome of an organism for the annotation purpose.

Introduction:
MitPred is a stand-alone program of web-server specifically trained to predict the proteins which are destined to localize in mitochondria in yeast and animals particularly. The prediction is made on basis of either occurrence of Pfam domain(s) or homology to an experimentally annotated proteins or *ab-initio* prediction on the basis of amino acid composition. Domain search is being done my HMMER (hidden Markov Models based search) while homology search by BLAST. Since both of these methods rely on the presence of experimentally annotated examples which can be limiting in their absence, hence provision of SVM based prediction is also kept.

Programs needed to run Mitpred locally:

Main program, mitpred, present in bin folder of the package

Usage: perl mitpred -i inputFile -m model -t threshold -o Output_Result_File

-i inputFile (in Fasta format)
-m Model ('svm' for SVM based or 'blast' for BLAST based prediction or 'pfam' for Pfam based)
-t [optional] Threshold for SVM based [default =0.5]
E-value selected for Blast based model [default=1e-4]
-o Output Result file

Mitpred program is for the prediction of mitochondrial proteins. It offers 3 models/methods-

3. SVM based
4. Blast search + SVM based

5. Pfam search + SVM based

1) **SVM Based :** in this model query sequence (in Fasta format) leads to the following path-

Split amino acid composition is taken as a feature vector where query sequence is divide into 3 halves (split) and each part (n, rest and c) composition is calculated and given to SVM.

(For each program details see the package documentation)

9. bin/fasta2sfasta : present in the bin directory of the package to make multi-fasta format sequence into simple-fasta (SFASTA) format
10. bin/pro2aac_nt : calculate N terminal, 25 aa composition
11. bin/pro2aac_rest: calculate composition excluding n 25 and c 25
12. bin/pro2aac_ct : calculate composition C terminal, 25 aa
13. bin/add_cols : Add all 3 composition files (output of steps 2,3,4) in 2 steps creating matrix of 3*20 ie 60.
14. bin/col2svm: Converting this to SVM readable format
15. SVM classify with model svm_models/mitpred/model_file

2) **BLAST Search + SVM:**

Hybrid approach in which first query sequence is subjected to Blast against the mitochondrial and non-mitochondrial database 'mitp_dbase' (present in the blastdb/mitpred/ folder) and the result is parsed and checked for the top hits.

If blast returns positive or negative hit (as database sequences are already tagged as positive and negative), then Prediction directly assigns the query as mitochondrial or non-mitochondrial respectively. If blast returns no hit, then that query will be subjected to ab initio SVM prediction (by using above mentioned feature ie split amino acid composition).

Programs needed: (for each program details see the package documentation)

3. bin/fasta2sfasta : present in the bin directory of the package to make multi-fasta format sequence into simple-fasta (SFASTA) format
4. Convert each sfasta sequence to a fasta file as blast can take take one sequence at a time and in fasta format.
5. /blastall -p blastp -i inputSequence -e thresold -d /mitpred/mitp_dbase -o blast.out : Doing Blast
6. perl bin/take_blast_hit blast.out > take_blast_temp : to parse the blast output

7. If hit is positive or negative then declare the query as mitochondrial or non-mitochondrial respectively
8. If hit is No hit then Do SVM as above
9. Compare the predicted score with Threshold selected , If score is more or equal to threshold then Positive otherwise Negative

3) Pfam Search + SVM:

In this module query is first subjected to Pfam search by using hmmpfam program against a profile database (mitpred/mitpred_v2.hmm) of mitochondrial and non-mitochondrial domains created by hmmbuild. Then result is parsed and if hit domain is found, may be of mitochondrial or non-mitochondrial then declare this query as as mitochondrial or non-mitochondrial respectively.
If No domains or mitpred curated domains are found then SVM prediction is exploited same as above.

Programs needed: (for each program details see the package documentation)

6. /hmmpfam -E 1e-5 /mitpred/mitpred_v2.hmm inputFastaSeq >temp_pfam : Pfam search
7. perl bin/parse_result_hmm . > temp_hmm_parse_result : Parsing output
8. perl bin/domain_mitpred . > temp_domain : Domain assignment with /mitpred/domain.dat file

In domain assignment program searches for the hit domain in a file ie /mitpred/domain.dat where it's already classified that which domain is present in mitochondrial and non-mitochondrial or which are shared domains.

With shared or No Domain found results program does SVM as above.

Reference:
Webserver: www.imtech.res.in/raghava/mitpred
Kumar M, Verma R, Raghava GPS. (2005) Prediction of mitochondrial proteins using support vector machine and hidden Markov model. J Biol Chem. 281:5357-63.

NpPred

Application:

Program NpPred is for the prediction of Nuclear and non-nuclear proteins. This program will aid in the annotation of uncharacterized proteins.

Introduction:
NpPred is a method developed for predicting nuclear proteins. This method has been developed on a non-redundant dataset consists of 2710 nuclear and 7662 non-nuclear proteins. During development of NpPred we developed number of SVM based methods using various types of composition (amino acid, dipeptide, split) and achieved maximum accuracy 85.47% when evaluated using fivefold cross-validation. Using hybrid approach (Pfam domain and SVM) accuracy increased to 94.61%. This method performed better than existing methods when evaluated on independent dataset obtained from BaCelLo (Pierleoni et al., 2006) and NucPred (Brameier et al., 2007). In this server we have given 2 approaches for prediction (a) SVM module developed using N-terminal 25 and remaining residues amino acid composition and (b) Hybrid approach combining SVM module and HMM profile. We hope this method will be useful for researcher working on field of genome annotation.

Programs needed to run NpPred locally:

Main program, nppred, present in bin folder of the package

Usage: perl bin/nppred -i inputFile -m model -t threshold -o Output Result File

-i inputFile (in Fasta format)
-m [optional] Model ['svm' for SVM based or 'pfam' for Pfam based]
-t [optional] Threshold for SVM based [default =0.5]
-o Output Result file

NpPred program is for the prediction of nuclear proteins. It offers 2 models/methods-

6. SVM based
7. Pfam search + SVM based

1) **SVM Based :** in this model query sequence (in Fasta format) leads to the following path-

Split amino acid composition is taken as a feature vector where query sequence is divide into 3 halves (split) and each part (n, rest and c) composition is calculated and given to SVM.

(For each program details see the package documentation)

16. bin/fasta2sfasta : present in the bin directory of the package to make multi-fasta format sequence into simple-fasta (SFASTA) format
17. bin/pro2aac_nt : calculate N terminal, 25 aa composition
18. bin/pro2aac_rest: calculate composition excluding n 25
19. bin/add_cols: Add 2 composition files (output of steps 2,3) creating matrix of 2*20 ie 40.
20. bin/col2svm: Converting this to SVM readable format
21. SVM classify with model svm_models/nppred/model_file

2) Pfam Search + SVM:

In this module query is first subjected to Pfam search by using hmmpfam program against a profile database (nppred/nppred.hmm) of mitochondrial and non-mitochondrial domains created by hmmbuild.

Then result is parsed and if hit domain is found, may be of Nuclear or non-nuclear then declare this query as as Nuclear or non-nuclear respectively.

If No domains or nppred curated domains are found then SVM prediction is exploited same as above.

Programs needed: (for each program details see the package documentation)

10. /hmmpfam -E 1e-5 /nppred/nppred.hmm inputFastaSeq >temp_pfam : Pfam search
11. perl bin/parse_result_hmm . > temp_hmm_parse_result : Parsing output
12. perl in/domain_nppred . > temp_domain : Domain assignment with /nppred/domain.dat file

In domain assignment program searches for the hit domain in a file ie /nppred/domain.dat where it's already classified that which domain is present in nuclear or non-nuclear or which are shared domains.

With shared or No Domain found results program does SVM as above

Reference:

http://www.imtech.res.in/raghava/nppred/

Kumar, M. and Raghava, G.P.S. Prediction of Nuclear Proteins using SVM and HMM

Models. BMC Bioinformatics. 2009 Jan 19; 10(1):22.

Pprint

Application:

There are many proteins or factors which are involved in the gene regulation process and work very efficiently. These factors are important areas of research in modern biology due to direct application in many diseases. The present program address the very common question asked by a biologist that what are the residues interacting with RNA in a RNA interacting proteins.

Introduction:

Pprint (Prediction of Protein-RNA Interaction) is a stand-alone version of a web-server for predicting RNA-binding residues of a protein. The prediction is done by SVM model trained on PSSM profile generated by PSI-BLAST search of 'swissprot' protein database. The SVM model is trained and tested on a set of 86 non-homologous protein chains with 5-fold cross-validation. It has predicted RNA-interacting amino acids with prediction accuracy 75.53% and MCC value of 0.44 during training and testing. It takes amino acid sequence in FASTA format as input and predict the RNA-interacting residues. The residues in the query sequence predicted as RNA-interacting residues are printed in Upper case and non-interacting residues are in lowercase. Below the amino acid sequence, residue-wise detail prediction is also given in tabular format. This table contains three columns (i) amino acid residue, (ii) SVM score and (iii) prediction. The prediction result depends on the threshold value specified by the user. The default threshold is set as -0.2. To get prediction with less number of false positives, the user should choose higher threshold. For prediction with less number of false negatives, threshold should be very low.

Usage:

perl bin/pprint -i inputFile -t threshold -o Output Result File
-i inputFile (in Fasta format)
-t [optional] Threshold for SVM based Prediction [default = -0.2]
-o Output Result file

Programs Needed:

For running pprint user need following programs- (for details of each program see the

program manual)

pprint: Main running Program present in the bin directory of the package

8. bin/fasta2sfasta : present in the bin directory of the package to make multi-fasta format sequence into simple-fasta (SFASTA) format (for details see the package documentation)
9. bin/seq2pssm_imp_pprint : program to generate PSSM matrix from sequences
10. bin/pssm2pat_pprint : program to generate defined length patterns from pssm matrix
11. bin/col2svm : for converting pssm column values to svm readable format
12. model_pprint : present in /svm_models/pprint/ folder , to run SVM classify and get predicted scores
13. Compare the predicted score with threshold selected for each motif and predict Interacting or Non-interacting

Sample output

pprint:: Prediction of RNA-interacting residues Result ## No of sequences = 2 ## Threshold = -0.2

Lowercase: Interacting residues Uppercase: Non-interacting residues

>1AF3_B_PDBID_CHAIN_SEQUENC Length = 196 amino acids
msqSNRELVVDFlSykLSqKgySwSQFSDVEENRTEAPEETEPERETPSAINGNPSWH
LADSPAVNGATGHSSSLDAREVIPMAAVKQALREAGDEFELRYRRAFSDLTSQL
HITPGTAyQSFEQVVNELFrdgvNwGrIVAFFSfGGALCVESVDKEMQVLVSRIASW
MATYLNDHLEPwIQengGWDTFVDLYG

Residue wise detail prediction

Amino Acid	SVM Score	Prediction
M	0.054416444	Interacting
S	-0.024923589	Interacting
Q	-0.16793406	Interacting
S	-0.66043539	Non-Interacting
N	-0.55410943	Non-Interacting
R	-0.29945995	Non-Interacting
E	-1.0637555	Non-Interacting

```
L             -0.40939491    Non-Interacting
V             -0.72646616    Non-Interacting
V             -1.5926368    Non-Interacting
D             -0.90241588    Non-Interacting
F             -0.61350159    Non-Interacting
L             0.72456004     Interacting
S             -0.39280286    Non-Interacting
Y             -0.084172651   Interacting
K             0.91123144     Interacting
L             -0.74135724    Non-Interacting
. . . . . . . .. . . . . . . . . . . . .. . . . .  . ..  . . . .. . . . .  . .. . . . . .. . . .. .
```

Reference:

www.imtech.res.in/raghava/pprint

Kumar, M., Gromiha, M.M. and Raghava, G.P.S. Prediction of RNA binding sites in a protein using SVM and PSSM profile. Proteins: Structure, Function and Bioinformatics. 2008 Apr; 71(1):189-94.

SPpred

Application:

Solubility of protein is an important issue while doing the protein over expression studies in *Escherichia coli* because heterologous proteins may or may not be soluble enough to show activity or may result in to protein aggregates. Therefore a computational tool SPpred has been developed to predict the solubility of any protein before going into the real experimentation.

Introduction:

SPpred (Protein Solubility prediction), a program for predicting solubility of a protein on over expression in Escherichia coli. The prediction is done by SVM model based on splitted amino acid composition. The SVM model is trained and tested on a set of 192 proteins with 5-fold cross-validation. The prediction accuracy and MCC value are ~75% and 0.504 respectively during training and testing.
It takes amino acid sequence in FASTA format as input and predicts whether the given protein is soluble or form inclusion body on over expression. The prediction result depends on the threshold value specified by the user. The default threshold is set as -0.1. To get prediction with less number of false positives, the user should choose higher threshold. For prediction with less number of false negatives, threshold should be very low.

Method:
1) First query sequence is splitted into 4 parts.
2) Amino acid composition of each part is calculated.
3) All 4 feature vectors are added making it to vector size=80.
4) By using Col2svm program converted to SVM readable format, and then given to SVM for classification.
5) Based on the prediction score and threshold selected by the user prediction is done.

Usage:
perl bin/sppred -i <i/p file> -t <threshold> -o <o/p file>
-i Input Sequence in FASTA format
-t SVM threshold
-o Output Result File
Reference
http://www.imtech.res.in/raghava/sppred/

ISSPred

Application:

ISSPred program is for the identification of intein (protein splicing) and their N-C teminal splice site. Program has 3 different modules for the classification of Intein and non-intein containing proteins, Intein domain and their splice sites.

Introduction:
Protein Post-translational Modification (PTM) is a common phenomenon in biology which regulates the function of proteins. Protein Splicing is a unique PTM in that it leads to cleavage of protein into internal (intein domain) and flanking (extein domain) fragments. Extein sequences later ligate together to form fully functional active protein. Identification of intein and their splice sites aid in the annotation of uncharacterized proteins. In this study, attempts have been made to predict intein proteins, domains, and their sites. In order to predict Intein proteins, we analyzed amino acid composition of intein proteins/domain and observed preference for certain type of residues. Support Vector Machine (SVM) models have been developed for predicting intein proteins using amino acid and dipeptide composition and achieved maximum MCC 0.63 and 0.77 respectively. Secondly SVM models have been developed for predicting intein domains in protein using amino acid and dipeptide composition and achieved maximum MCC 0.76 and 0.87 respectively. Finally SVM models were developed for predicting splice sites using different window length and achieved maximum MCC 0.87 and 0.93 for N-splice and C-splice sites respectively. This study is the first attempt to predict intein proteins, domains and their splice sites. Based on above models a prediction server ISSPred has been developed, which is available at http://www.imtech.res.in/raghava/isspred/.

Usage:
perl isspred -i inPutFile -p prediction -m model -t threshold -o outPutFile

-i InputFile (multi-fasta format)
-p Prediction [d|p|s]
['d' for intein domain; 'p' for Intein-protein and 's' for Inteins's N-C Splice Site Prediction]
-m [optional] Model to use [a|d|n|c|nc][Default is 'd' if -p= d or p and 'nc' if -p is 's'] ['a' amino acid composition; 'd' dipeptide composition if -p is 'd' or 'p']
['n'= N splice, 'c'= C splice and 'nc' = both NC splice site prediction if -p is 's']
-t [optional] Threshold selected e.g -1.0 to +1 [default is -0.4 if -p= d ; -0.6 if -

p= p and -0.9 if -p= s]
-o Output result File

Program description: (for detailed description see the package documentation)
ISSPred is different from most of other programs in that it predicts the splice site (ie between two amino acid residues) unlike the center amino acid residue prediction used in Pprint, NADbinder etc.

ISSPred has 3 modules-

Amino acid composition:

This feature has been used for both Intein domain and Intein Protein prediction.

1) bin/pro2aac -i seq.sfasta -o seq.comp (sequence to amino acid composition)
2) bin/col2svm -i seq.comp -o seq.svm -s 0 (column to SVM Readable format)
3) svm_classify seq.svm svm_models/isspred/model_dipep_prot seq.pred > svmlog.out
4) bin/count_pred_isspred -s seq.sfasta -p seq.pred -o outPutFile -t thresold

Dipeptide composition:

Feature has been used for both Intein domain and Intein Protein prediction.

1) bin/pro2dpc -i seq.sfasta -o seq.aac (sequence to Dipeptide composition)
2) bin/col2svm -i seq.comp -o seq.svm -s 0 (column to SVM Readable format)
3) svm_classify seq.svm svm_models/isspred/model_dipep_prot seq.pred > svmlog.out
4) bin/count_pred_isspred -s seq.sfasta -p seq.pred -o outPutFile -t thresold

Splice (N or C) binary patterns:

Feature has been used in N and C terminal Splice site prediction.
1) bin/seq2motif -i seq.sfasta -w 16 -o seq.motif (sequence to motif)
2) bin/motif2bin -i seq.motif -x n -o seq.bin (motif to binary)
3) bin/col2svm -i seq.bin -o seq.svm -s 0 (column to SVM readable format)
4) svm_classify seq.svm /svm_models/isspred/model_nsplice seq.pred >svmlog.out
5) /bin/count_pred_binary -p seq.pred -s seq.sfasta -m seq.motif -o outPutFile -t thres -a N- Splice

Reference

http://www.imtech.res.in/raghava/isspreda

GSTPred

Application:
GSTPred is a standalone package for Glutathione S-transferase protein (GST) prediction webserver GSTPred. GSTPred trained using generalized GST proteins datasets can be used for the prediction of GST proteins. The webserver is available at http://www.imtech.res.in/raghava/gstpred/

Introduction:
Glutathione S-transferases (GSTs) are a group of ubiquitous and multifunctional enzymes found in both prokaryotes and eukaryotes. Another important function of GSTs are making a cell drug resistant by avoidance of apoptotic cells death, altered expression of multi-drug resistance-associated proteins or drug metabolism or uptake, and/or over-expression of GSTs. GSTs are involved in drug resistance by either i) participation in detoxification process with GSH or ii) increasing the pumping out of drug molecule from the cell or iii) inhibition of MAP kinase pathways. Overexpressions of specific GSTs in mammalians cells cause anti-cancer drug (alkylating agent used in cancer chemotherapy) resistance. First time we developed model for predicting GST proteins using SVM.

Datasets:
All sequences used in this study were downloaded from Swissprot database. All proteins were manually examined to retain only sequences, which have high quality annotation. For this we removed all sequences that was labeled as 'fragment' or annotated as putative or by similarity. We got total 137 proteins, which were experimentally annotated as 'GST protein'. The sequence redundancy of dataset was further removed by using CD-HIT such that no two proteins have sequence identity more than 90%. The final dataset contains total 107 GST protein sequences. Negative dataset was compiled by randomly selecting 107 proteins keeping in mind that they were experimentally annotated as non-GST protein and they didn't have sequence identity more that 90%. Here we are trying to developed broad-spectrum method for GSTs prediction hence we used both prokaryotes and eukaryotes (plant, fungi, animals) proteins in our study.

Results: We have used a dataset of GST and non-GST proteins for training and the performance of the method was evaluated with five-fold cross-validation technique. First a SVM based method has been developed using amino acid and dipeptide composition and achieved the maximum accuracy of 91.59% and 95.79% respectively. In addition we developed a SVM based method using tripeptide composition and achieved maximum accuracy 97.66% which is better than accuracy achieved by HMM based searching

(96.26%). Based on above study a web-server GSTPred has been developed

Usage of standalone version

perl gstpred -i <inputFile> -t <threshold > -m <mode> -o <output_Result_File>

-i inputFile (in Fasta format)
-m mode (monopeptide, dipeptide or tripeptide composition based)
-t Threshold for SVM based [default = 0]
-o Output Result file

GSTPred program: In this query sequence (in Fasta file format) leads to the following path

```
gpsr_home/bin/fasta2sfasta -i $input_file -o gst.sfa
gpsr_home/bin/pro2dpc -i gst.sfa -o gst00.aac
gpsr_home/bin/col2svm -i gst00.aac -o gst01.svm -s 0
gpsr_svm_classify  gst01.svm gpsr_home/src/svm_models/gstpredmodel gst01.pr
```

Publication: Nitish Mishra, Manish Kumar, Dr. G. P. S. Raghava Support Vector Machine based Prediction of Glutathione S-transferases. Protein and Peptide Letters. 14 (6), 2007, pp. 575-580

TBPred

Application:

The program is able to classify any query protein of mycobacterium sp. into any four different localizations. Stand-alone version is very useful for running whole proteome of an organism for the annotation purpose.

Introduction:

TBPred is a stand-alone program of web-server specifically trained to predict the subcellular locations of mycobacterial proteins. There are four methods to predict the locations, namely amino acid composition, dipeptide composition, Position Specific Scoring Matrix (PSSM), and Hybrid method. In hybrid method the result of MAST (motif alignment and search tool) has been given preference and if no hit comes from MAST, the decision is taken on the basis of PSSM composition. TBPRED predicts a protein into any of four locations namely, cytoplasmic, integral membrane, secretory, and membrane attached by lipid-anchor.

Programs needed to run TBpred locally:

Main program, tbpred, present in bin folder of the package

NOTE: tbpred program requires MEME/MAST software in addition. While during installation user must provide the local path of the respective softwares or tools.

Usage: perl tbpred -i inputFile -m method -o Output_Result_File –e E-valueThreshold

-i inputFile (in Fasta format)

-m Method
<A> for Amino acid composition based method
<D> for Dipeptide composition based method
<P> for PSSM composition based method
<H> for hybrid (MEME/MAST and PSSM) based method

-o Output Result file

-e E- value threshold for Hybrid based method [default =0.001]
Applied only when –m <H> is used.

tbpred program is for the prediction of mycobacterial proteins' locations. It offers 4 different SVM based models/methods-

Amino acid composition
Dipeptide composition
PSSM composition
Hybrid method

Following are the programs used to run to complete the prediction-

1) Amino acid composition

(For each program's detail see the package documentation)

- **bin/fasta2sfasta :** program to make fasta format into single fasta format
- **bin/pro2aac :** calculate whole protein amino acid composition
- **bin/col2svm:** Converting composition file to SVM readable format
- SVM classify with model **svm_models/tbpred/model_file**

2) Dipeptide composition

(For each program details see the package documentation)

- **bin/fasta2sfasta :** program to make fasta format into single fasta format
- **bin/pro2aac :** calculate whole protein amino acid composition
- **bin/col2svm:** Converting composition file to SVM readable format
- SVM classify with model **svm_models/tbpred/model_file**

3) PSSM composition

- **bin/fasta2sfasta :** program to make fasta format into single fasta format
- **bin/seq2pssm_imp :** calculate position specific scoring matrix for the protein
- **bin/pssm_n1 :** normalization of the scores got from seq2pssm_imp
- **bin/pssm_comp :** for each protein seq it forms a fixed length composition pattern of 400
- **bin/col2svm:** Converting composition file to SVM readable format
- SVM classify with model **svm_models/tbpred/model_file**

4) Hybrid method (MAST/PSSM)

- **mast program**

If mast is unable to classify at a given E-value threshold, then decision is taken from PSSM composition based method.

Reference:

Webserver: www.imtech.res.in/raghava/tbpred

Citation:
Rashid M, Saha S, Raghava GPS (2007): **Support Vector Machine-based method for predicting subcellular localization of mycobacterial proteins using evolutionary information and motifs.** *BMC Bioinformantics*, 8:337.

PSEAPred2

Application:
The program is able to classify *Plasmodium falciparum* query protein into Secretory or Non-secretory in localization. Stand-alone version is very useful for running whole proteome of an organism for the annotation purpose.

Introduction:
PSEAPred2 is a stand-alone program of web-server specifically trained to predict the *Plasmodium falciparum* proteins which are destined as secretory or non-secretory protein. The prediction is made on basis of support vector machine [SVM] based and motif based.

Usage of standalone version
perl pseapred2 -i inputFile -t threshold -o Output_Result_File

-i inputFile (in Fasta format)

-t Threshold for SVM based [default =0.0]

-o Output Result file

Pseapred2 program is for the prediction of secretory proteins. It predicts on 2 methods
Motif based
SVM based

Motif based: This model runs on MAST software, which takes a file in fasta input and gives a mast output file.

SVM based: in this model query sequence (in Fasta format) leads to the following path. The results are given on 3 models.
Programs needed: (for each program details see the package documentation)

bin/fasta2sfasta: present in the bin directory of the package to make multi-fasta format sequence into single fasta.
bin/pro2dpc -i file_sfasta -o comp_aa
bin/pro2aac_split -i file_sfasta -o comp_split -n3
bin/col_mult.pl -i comp_split -o comp_split_main -n 0.01
bin/add_cols -i comp_aa -c comp_split_main -o comp_final
bin/col2svm -i comp_final -o comp_svmpat -s 0
SVM classify with model svm_models/pseapred2/model-hyb svm_temp > svm.out

Subsequent steps followed: (model2)

```
bin/pro2aac -i file_sfasta -o comp_aa1
bin/col_mult.pl -i comp_aa1 -o comp_aa1_final -n 0.01
bin/col2svm -i comp_aa1_final -o comp_svmpat1 -s 0
SVM classify with model svm_models/pseapred2/main-model svm_temp1 > svm1.out
```

Model3:
/bin/pro2aac -i file_sfasta -o comp_aa11
/bin/col_mult.pl -i comp_aa11 -o comp_aa11_final -n 0.01
/bin/col2svm -i comp_aa11_final -o comp_svmpat11 -s 0

Reference:

Webserver: www.imtech.res.in/raghava/pseapred2

List of Contributers

These methods have been developed over the years. Although it is not possible to include name of all but major contributers are Harpreet Kaur Saini, Biju Issac, Manoj Bhasin, Sudipto Saha, Manish Kumar, Aarti Garg, Sneh Lata, Mamoon Rashid, Nitish Kumar Mishra, Firoz Ahmed, Hifzur Rahman Ansari Ruchi Verma, Deepti Sethi, and Yogita Sharma.

Group members involved in integration and documentation of GPSR package are Aarti Garg, Hifzur Rahman Ansari, Nitish Kumar Mishra, Firoz Ahmed, Sneh Lata, Mamoon Rashid, Harinder Singh, Jasjit Kaur, Jagat S. Chauhan, Deepak Singla, Bharat Panwar, Arun Sharma, Ravi Kumar, and Sandhya Agarwal.

About G P S Raghava

Raghava is a scientist working at Bioinformatics Centre, Institute of Microbial Technology (IMTECH), Chandigarh, India. He did M.Sc. form Meerut University, M.Tech from IIT Delhi and PhD from IMTECH Chandigarh.

He worked as Postdoctoral fellow at Oxford University UK (1996-98), Bioinformatics specialist at UAMS, USA (2002-3 & 2006) and visiting professor at POSTECH, South Korea (2004). His group developed more than 100 web servers, 100 research papers, 50 Copyrights, 10 databases and mirror sites. He is responsible for setting Bioinformatics infrastructure at IMTECH and at UAMS Little Rock, USA (http://bic.uams.edu/). He got following major awards/recognition I) Shanti Swarup Bhatnagar Award in Biological Science ii) "National Bioscience Award for Carrier Development" for year 2005-2006 (by Department of Biotechnology, Govt. India); iii) fellow of National Academy of Sciences (FNASc); and iv) fellow of Indian Academy of Science (FASc), Banglore.

Contact

G P S Raghava
Scientist and Head Bioinformatics Centre
Institute of Microbial Technology
Sector-39A, Chandigarh, India
Phone: +91-172-2690557, Fax: +91-172-2690632
http://www.imtech.res.in/raghava/
email: raghava@imtech.res.in