**Predict Remote Homology Proteins by Grey Incidence Analysis and Functional Domain Analysis**

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**Running Title: Remote Homology Protein detection**

**ABSTRACT**

Protein remote homology detection is a challenging problem for drug development. Although there are a couple of methods to deal with this problem, the benchmark datasets based on which the existing models were trained and tested contained many high homologous samples due to the fact that the cutoff threshold was set at 95%. In this study, we reconstructed the benchmark dataset by setting the threshold at 40%, meaning none of the proteins included has more than 40% pairwise sequence identity with any other. Using the new benchmark dataset, we proposed a new method called PHom-GRA to detect the remote homologous proteins by integrating various ranking approaches via grey relational analysis and functional domain analysis. Rigorous cross-validations have indicated that the new predictor is superior to its counterparts in both enhancing successes rates and reducing computational cost.

**1. INTRODUCTION**

Detecting remote homology relationship among proteins plays one of the fundamental and central roles in computational proteomics. It is particularly useful for drug development (see, e.g.,[[1](#_ENREF_1), [2](#_ENREF_2)]). With the development of sequencing techniques, the protein sequence data rapidly raise. To find those proteins structure and function is more and more urgent. Although X-ray crystallography is a powerful tool in determining protein 3D structures, it is time-consuming and expensive. Particularly, not all proteins can be successfully crystallized, particularly for membrane proteins. The NMR technique is indeed a very powerful tool in determining the 3D structures for membrane proteins as indicated by a series of recent publications (see, e.g., [[3-7](#_ENREF_3)]), it is time-consuming and costly. To acquire the structural information in a timely manner, one has to resort to various structural bioinformatics tools based on the sequence similarity principle (see, e.g., [[8](#_ENREF_8)]). Unfortunately, such principle cannot cover the cases of remote homology proteins. In view of this, considerable efforts [[9-14](#_ENREF_9)] have been made to detect remote homology proteins.

Although these methods each had their own merits and did play stimulating role in this area, further work is needed. Firstly, the benchmark datasets used in their studies had high similarity. For instance, the benchmark dataset in [[9](#_ENREF_9), [12](#_ENREF_12)] contains 7329 proteins from 1070 different super families, with pairwise sequence identity cutoff set at 95%. In other words, it would allow those proteins with higher than 80% similarity in the data set. Secondly, the ranking algorithm used in those studies would spend a lot of time to training the learning model. For example, if the training dataset has N proteins, the LambdaMART need to deal with N2 proteins pair samples.

The present study was initiated to address the two problems with the aim to develop a more powerful method in this regard.

**2. MATERIALS AND METHOD**

**2.1 Benchmark Dataset**

According to Chou’s 5-step rules [[15](#_ENREF_15)] that have been widely and increasingly used by many investigators (see, e.g., [[16-32](#_ENREF_16)]), the first prerequisite in establishing a new predictor is to construct or select an effective benchmark dataset.

In this study, the benchmark dataset was taken from Liu et al. [[12](#_ENREF_12)]. It included 7329 proteins from 1070 different super families and 1824 families derived from SCOP database. To reduce the redundancy and homology bias, the program CD-HIT[[33](#_ENREF_33)] was adopted to cut down those proteins that had ≥40% pairwise sequence identity to any other in the dataset. Furthermore we removed those families that just had one protein sequence. Finally, we obtained 3128 proteins from 540 super-families and 777 families.

**2.2 Sample Similarity Analysis**

2.2.1 Grey Incidence Analysis of proteins formulated by Grey-PSSM

Given a protein with *L* amino acid residues, it is usually expressed by

|  |  |
| --- | --- |
|  | (1) |

where is the *i*-th residue in the protein. Since all the existing machine-learning algorithms can only handle vector but not sequence samples [[34](#_ENREF_34)], one has to convert Eq.1 into a vector model. But a biological sequence expressed as a vector in the discrete framework may lose all the sequence-order or pattern information.

To avoid completely losing this kind of information for proteins, the pseudo amino acid composition (PseAAC)[[35](#_ENREF_35), [36](#_ENREF_36)] was proposed. Ever since the concept of Chou’s PseAAC was proposed, it has been widely used in nearly all the areas of computational proteomics (see, e.g., [[37-44](#_ENREF_37)] as well as a long list of references cited in[[45](#_ENREF_45), [46](#_ENREF_46)]). According to the general PseAAC[[15](#_ENREF_15)], the protein of Eq.1 can be formulated as

|  |  |
| --- | --- |
|  | (2) |

where **T** is the transposing operator, the subscript is an integer, and its value and the components will depend on how to extract the desired features and properties from the protein sequence.

In this study, the model, Grey-PSSM proposed by Lin[[47](#_ENREF_47), [48](#_ENREF_48)], is adopted. It extracted the sequential evolution information by the Position Specific Scoring Matrix (PSSM). For the concrete procedures, refer to the original papers [[47](#_ENREF_47), [48](#_ENREF_48)].

After the Grey-PSSM treatment, we have finally got a 60-D PseKNC vector for Eq.2; i.e., its subscript parameter and each of the 60 components therein has been uniquely defined.

Assume

(3)

are the set of protein samples, and is the protein. According to Equals [6]~[11] in Ref. [[49](#_ENREF_49)], the distance is defined as the grey incidence degree between and . The larger the value of , the more similar they are.

2.2.2 Functional Domain Analysis

There are also other models used to formulating proteins excepte for the PseAAC. Here, we propose a noval mothed to discribe the proteins. For a protein , we discribe its functional domains by the following steps.

Step 1, the homology set of protein , , can be extracted by searching against UniProt release 2018\_08 Swiss-Prot FASTA format flatfile by HMMER[[50-52](#_ENREF_50)]. We just use the top 10 sequences if the search results have more than 10 sequences. Therefore there are at most 10 proteins in .

Step 2, for a protein , we annotate its functional domains by running hmmscan program against Pfam-A database (Pfam release 32.0). The Pfam-A includes 17,929 domains and 688 clans. Some functional domains have same clan. For example, the domains of “PF15884” and “PF17050” have the same clan “CL0683”. Therefore, the functional domains of protein is expressed as a set {| is included in Pfam-A database} .

Step 3, the protein is expressed as a domains set .

As aboved steps, a protein can be expressed a set including some functional domains. For the proteins in same family or clan have similar functional domains, new distance between two proteins, named as Functional Similarity Index (FSI), can be defined based on the functional domains.

The algorithm of distance between , is discribed as follows.

1) If,

2) Else If

| is the clan of each domain in }

| is the clan of each domain in }

2.1) If , . F is a constant and in this study F is equal to 0.2.

2.2) Else,

From above discription, we have and the larger the , the more similarity they are.

**2.3 Operation Engine or Algorithm**

In this study, the Grey Relational Analysis [[53](#_ENREF_53), [54](#_ENREF_54)] and the Functional Similarity Index was utilized to rank the relationship of proteins. Given a query protein, the system will search it against the benchmark dataset and return the top ranked proteins. The predictor thus formed is called “PHom-GRAFSI”. Illustrated in **Figure 1** is a flowchart to show how the proposed predictor is working.

**3. RESULT AND DISCUSSION**

The jackknife test is deemed the least arbitrary and most objective among three cross-validation methods: independent dataset test, K-fold cross-validation test and jackknife test [[55](#_ENREF_55)]. Because the LambdaMART ranking algorithm used in preview studies [[9](#_ENREF_9), [12](#_ENREF_12)] consumed more training time and computer memory, as a compromise the 5-fold cross-validation test was adopted there. Now, we employed GRA and FSI to compute the relationship score between the query protein and benchmark dataset proteins, significantly reducing the computing time and memory. Therefore it would be feasible to use the most rigorous jackknife test to examine the prediction quality. The outcome thus obtained are given in **Table 1**, where we can see that PHom-GRAFSI achieved the best performance in both the score of ROC1 and the score of ROC50.

**4. CONCLUSION**

Protein remote homology detection is vital for studying protein structures and functions. It is anticipated that the proposed method may become a useful high throughput toll for both basic research and drug design.

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**Table 1**. A comparison of the jackknife test results for protein remote homology detection on the benchmark dataset

|  |  |  |
| --- | --- | --- |
| Methods | ROC1 | ROC50 |
| PSI-BLAST | 0.7113 | 0.7647 |
| GRA(Grey-PSSM) | 0.8937 | 0.7149 |
| FSI | 0.9053 | 0.8454 |
| PHom-GRAFSI | 0.9620 | 0.8861 |

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