IDENTIFYING THE NOVEL DOMAIN INVOLVED IN HUMAN PATHOGENESIS

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ABSTRACT

Functional prediction of human hypothetical proteins remains a major project for biologist to carry out their further research in various life science and healthcare segments. Human chromosome 1 is the one which is very largest and has many hypothetical proteins. So it is attempted to find the function of some of the human chromosome 1 hypothetical proteins, the proteins are clustered based on their sequence similarities and identify the consensus sequence in those proteins. Using various prediction methods their functional annotations are carried out and a domain was predicted which is involved in the pathogenic functionality.

Keywords: *Function Prediction, Hypothetical Protein, Consensus Sequence, Domain.*

1. INTRODUCTION

When genetics was new, time-consuming lab experiments were the only way to determine what proteins did. But, now the functional genomics can able to determine the function of genes and proteins as a means to understand life, health and illness. Currently, most approaches to protein function prediction rely on searching sequence databases for homologous sequences with prior annotation. However, the function for one protein cannot be inferred from another when similarity is <40% sequence identity. Moreover, studies on enzyme proteins have shown that the precise function diverges below identities of 60%.

. The following are the general classes of function prediction:

- Sequence based approaches
- Structure-based approaches
- Motif-based approaches

The detection of function-related local 3D-motifs in protein structures can provide insights towards protein function in absence of sequence or fold similarity. Protein loops are known to play important roles in protein function and several loop classifications have been described, but the automated identification of putative functional 3D-motifs in such classifications has not yet been addressed. This identification can be used on sequence annotations

Most of the work on predicting protein function uses a single source of in-formation – the most common being the amino acid sequence of the protein(see Chapter 30). There are, however, a number of sources of data that are predictive of protein function.

The general categories of function are:

i) **Information:** storage, maintenance of the genetic code; DNA replication/repair; general transcription/translation.
ii) **Regulation:** regulation of gene expression and protein activity; information processing in response to environmental input; signal transduction; general regulatory or receptor activity.
iii) **Metabolism:** anabolic and catabolic processes; cell maintenance/homeostasis; secondary metabolism.
iv) **Intra-cellular processes:** cell motility/division; cell death; intra-cellular transport; secretion.
v) **Extra-cellular processes:** inter-, extra-cellular processes, e.g. cell adhesion; organismal processes, e.g. blood clotting, immune system.
vi) **General:** general and multiple functions; interactions with proteins/ions/lipids/small molecules.
vii) **Other/Unknown:** unknown function, viral proteins/toxin.

2. HYPOTHETICAL PROTEINS OF HUMAN CHROMOSOME 1:

Genes with unknown function are called orphan genes while their transcripts and peptides are called hypothetical proteins. There are many genes and their associated proteins that remain uncharacterized in the human genome.

In biochemistry, a hypothetical protein is a protein whose existence has been predicted, but for which there is no experimental evidence that it is expressed in vivo. The usual scenario involving a hypothetical protein is in gene identification during genome analysis. When the bioinformatics tool used for the gene identification finds a large open reading frame without an analog in the protein database, it returns "hypothetical protein" as an annotation remark.

There are many hypothetical proteins in Human chromosome1 whose function is not yet identified. Chromosome 1 is the designation for the largest human chromosome. People normally have two copies of chromosome 1, as they do with all of the autosomes, which are the non-sex chromosomes. Chromosome 1 spans about 247 million nucleotide base pairs, which are the basic units of information for DNA. It represents about 8% of the total DNA in human cells. Identifying genes on each chromosome is an active area of genetic research. Chromosome 1 is currently believed to have 4,220 genes, exceeding previous predictions based on its size. It was the last completed chromosome, sequenced two decades after the beginning of the Human Genome Project. The number of single nucleotide polymorphisms (SNPs) is about 740,000.

3. LITERATURE REVIEW:

Prediction of protein function from sequence and structure is a difficult problem, because homologous proteins often have different functions. Many methods of function prediction rely on identifying similarity in sequence and/or structure between a protein of unknown function and one or more well-understood proteins. Alternative methods include inferring conservation patterns in members of a functionally uncharacterized family for which many sequences and structures are known. However, these inferences are tenuous. Such methods provide reasonable guesses at function, but are far from foolproof.

The computational assignment of protein function may be the single most vital application of bioinformatics in the post-genome era. These assignments are made based on various protein features, where one is the presence of identifiable domains.

Protein domains, the building blocks of all globular proteins, are units of compact three-dimensional structure as well as of molecular evolution. The growth of sequence databases has elevated the need for computational annotation of proteins, and the detection of conserved domains is one of the first steps towards assigning molecular function.

Domains vary in length from between about 25 amino acids up to 500 amino acids in length. The shortest domains such as zinc fingers are stabilized by metal ions or disulfide bridges. Domains often form functional units, such as the calcium-binding EF hand domain of calmoduin. Because they are self-stable, domains can be "swapped" by genetic engineering between one protein and another to make chimera proteins.

The concept of the domain was first proposed in 1973 by Wetlaufer after X-ray crystallographic studies of hen lysozyme, papain and by limited proteolysis studies of immunoglobulins. Wetlaufer defined domains as stable units of protein structure that could fold autonomously.

In the past domains have been described as units of:

- Compact structure.
- Function and evolution.
- Folding.

Each definition is valid and will often overlap, i.e. a compact structural domain that is found amongst diverse proteins is likely to fold independently within its structural environment. Nature often brings several domains together to form multidomain and multifunctional proteins with a vast number of possibilities. In a multidomain protein, each domain may fulfill its own function independently, or in a concerted manner with its neighbors. Domains can either serve as modules for building up large assemblies such as virus particles or muscle fibres, or can provide specific catalytic or binding sites as found in enzymes or regulatory proteins.

Covalent association of two domains represents a functional and structural advantage since there is an increase in stability when compared with the same structures non-covalently

associated. Other, advantages are the protection of intermediates within inter-domain enzymatic clefts that may otherwise be unstable in aqueous environments, and a fixed stoichiometric ratio of the enzymatic activity necessary for a sequential set of reactions.

4. MATERIALS AND METHODS:

Literature Review has been proven that the protein function prediction will be more accurate once the domain is identified.

Step 1:

The hypothetical protein sequence of human chromosome 1 is collected from the NCBI database. Though there are many protein database, NCBI is widely used, because it will develop new information technologies to aid in the understanding of fundamental molecular and genetic processes that control health and disease.

Step 2:

The collected hypothetical proteins are clustered by means of ClustalW tool. Multiple alignments of protein sequences are important tools in studying sequences. The basic information they provide is identification of conserved sequence regions. This is very useful in designing experiments to test and modify the function of specific proteins, in predicting the function and structure of proteins, and in identifying new members of protein families.

Sequences can be aligned across their entire length (global alignment) or only in certain regions (local alignment). This is true for pairwise and multiple alignments. Global alignments need to use gaps (representing insertions/deletions) while local alignments can avoid them, aligning regions between gaps.

ClustalW is a fully automatic program for global multiple alignment of DNA and protein sequences. The alignment is progressive and considers the sequence redundancy. Trees can also be calculated from multiple alignments. The program has some adjustable parameters with reasonable defaults. It calculates the best match for the selected sequence, and lines them up so that the identities, similarities and differences can b seen. Evolutionary relationships can be seen via viewing phylograms or dendrograms.

Step 3:

Using the PFP server, the function of hypothetical proteins can be predicted. PFP is designed to predict Gene Ontology annotations for a query protein sequence beyond what can be found by searching conventional databases. The PFP algorithm has been shown to increase coverage of sequence-based function annotation more than fivefold by extending a PSI-BLAST search to extract and score GO terms individually and include information from distantly related sequences. It applies a novel data mining tool, the Function Association Matrix (FAM), to score significantly associating pairs of annotations.

The current version of the prediction method uses a sequence-based method. The aim is to utilize information from relatively weak hits in PSI-BLAST, which are not conventionally used. Typically, weak hits in PSI-BLAST are not perfect orthologs to the query sequence, but rather share a common functional domain. In addition to simply transferring the function of the common domain to the query sequence, the idea is to also consider those functions which are frequently associated with the annotated functions of the domain. To this end, it has built Function Association Matrices (FAMs) that quantify the co-occurrence of Gene Ontology (GO) annotations in sequences of the UniProt database. The GO is a controlled hierarchical vocabulary describing the function of genes in three categories: function, process, and component. Approximately two thirds of associated function pairs mined from UniProt bridge functions of different categories. Thus, it can assign function using FAMs that cannot be retrieved directly from highly similar sequences or structures.

The results are listed as the top ten most probable Gene Ontology annotations in the Biological Process, Molecular Function, and Cellular Component categories. It should be note that these automated function predictions are not intended to be perfectly accurate, but only to represent the statistical probability that your sequence matches the listed function annotations according to known associations extracted from a variety of publicly available functional databases. These results were cross-referenced to the GO database version GO_200702 to create a list of primary annotations for query sequence.

Step 4:

There are some secondary structure prediction tools which are commonly used to predict the secondary structure of protein domain. They are:

SOPM:

The first step of the SOPM is to build sub-databases of protein sequences and their known secondary structures drawn from 'DATABASE.DSSP' by (i) making binary comparisons of all protein sequences and (ii) taking into account the prediction of structural classes of proteins. The second step is to submit each protein of the sub-database to a secondary structure prediction using a predictive algorithm based on sequence similarity. The third step is to iteratively determine the predictive parameters that optimize the prediction quality on the whole sub-database. The last step is to apply the final parameters to the query sequence.

SOPMA:

Recently a new method called the self-optimized prediction method (SOPM) has been described to improve the success rate in the prediction of the secondary structure of proteins. This improved SOPM method (SOPMA) correctly predicts 69.5% of amino acids for a three-state description of the secondary structure (alpha-helix, beta-sheet and coil) in a whole database containing 126 chains of non-homologous (less than 25% identity) proteins.

GOR IV:

The GOR method is based on information theory and was developed by J.Garnier, D.Osguthorpe and B.Robson (J.Mol.Biol.120, 97, 1978). The present version, GOR IV, uses all possible pair frequencies within a window of 17 amino acid residues. After cross validation on a data base of 267 proteins, the version IV of GOR has a mean accuracy of 64.4% for a three state prediction (Q3). The program gives two outputs, one eye-friendly giving the sequence and the predicted secondary structure in rows, H=helix, E=extended or beta strand and C=coil; the second gives the probability values for each secondary structure at each amino acid position. The predicted secondary structure is the one of highest probability compatible

SIMPA 96:

A secondary structure prediction algorithm is proposed on the hypothesis that short homologous sequences of amino acids have the same secondary structure tendencies. Comparisons are made with the secondary structure assignments of Kabsch and Sander from X-ray data [(1983) Biopolymers 22, 2577-2637] and an empirically determined similarity matrix which assigns a sequence similarity score between any two sequences of 7 residues in length. This similarity matrix differs in many respects from that of the Dayhoff substitution matrix.

5. METHODOLOGY:

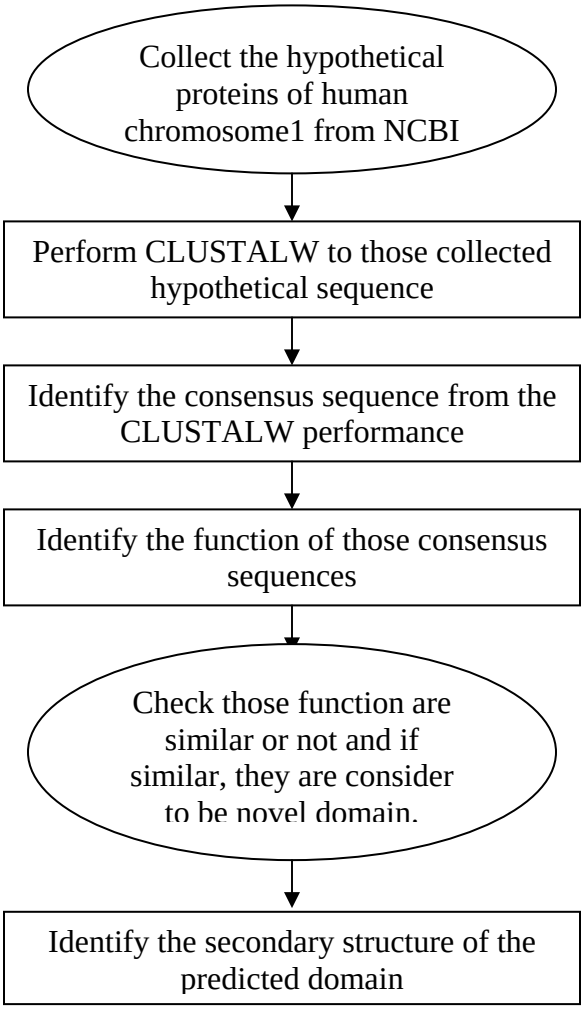


Figure 1: Methodology.

6. RESULTS AND DISCUSSIONS:

6.1) There are 180 hypothetical proteins of Human chromosome 1 in the NCBI database. They are retrieved.

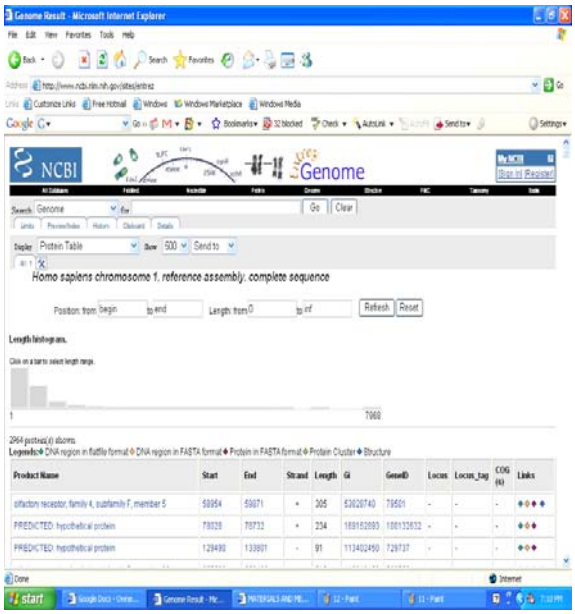
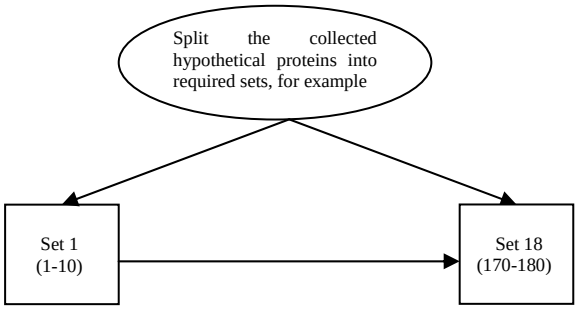
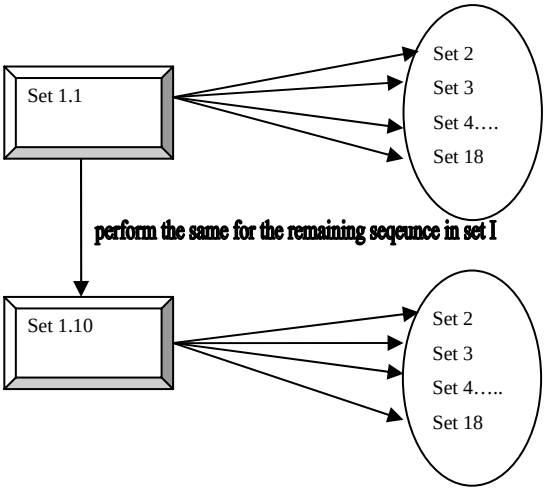


Figure 2: Hypothetical Proteins of Human Chromosome 1 in NCBI database.

6.2) The collected hypothetical proteins are submitted to the ClustalW program and perfoms the cluster as follows:



Like wise split all the 180 hypothetical protein and perform the ClustalW those sets, Here the example of clustering with Set I.



Similarly perform for the remaining sequence in all the set.

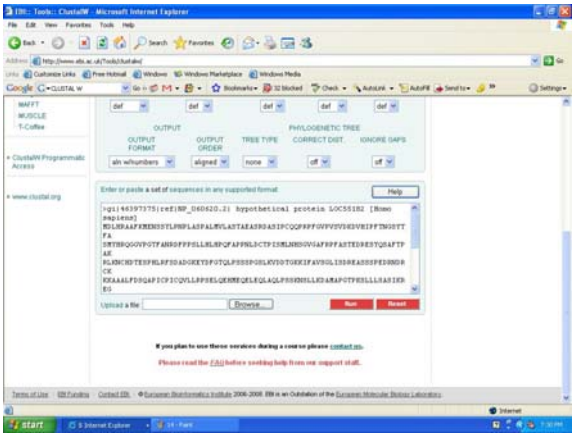


Figure 3: ClustalW Home Page.

From the number of ClustalW performance, the consensus sequence is identified and given below:

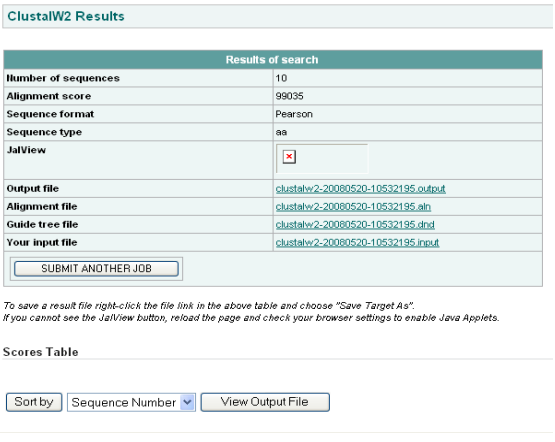
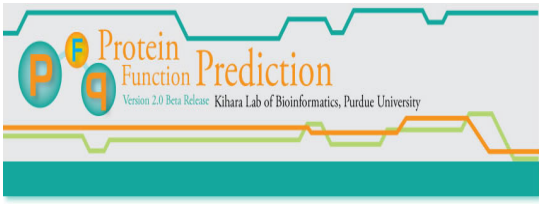


Figure 4: ClustalW Results, Yellow shading indicates the Conserved region.

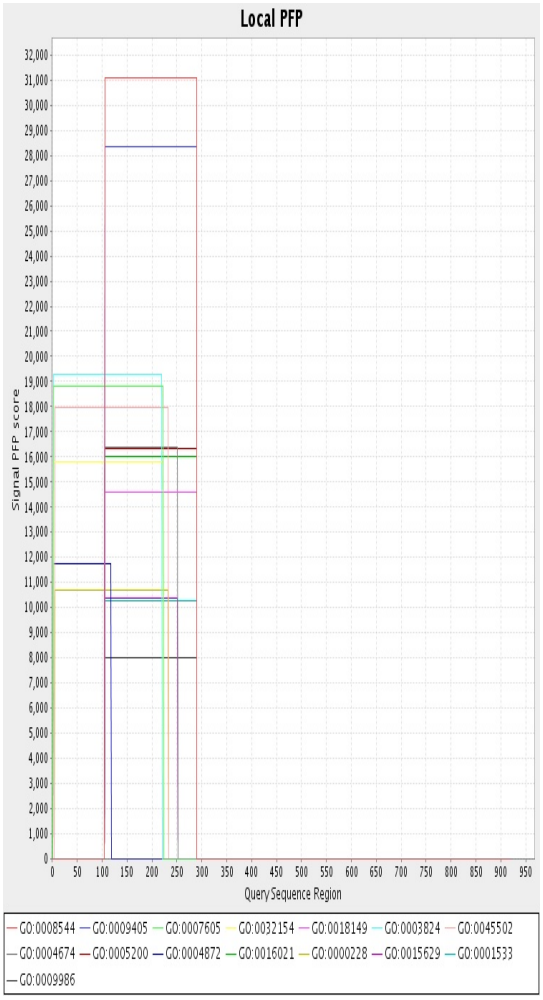
From the result of CLUSTALW, these ten sequences show the conserved region at **QYKVLVHAQERELTQLREKLREGRDASRS LNEHLQALLTPDEPDKSQGQDLQEQLAEG CRLTQHLVQKLSPENDNDDDEDVQVEVAE KVQKSSAPREMQKAEEKEVPEDSLEECAIT CSNSHGPYDSNQPHKKTKITFEEDKVDSTLI GS.**

This may be act as a common domain if they share the same functional property.

6.3) The sequences that are found have the conserved regions are submitted to the **protein function prediction server** and the functions are predicted for those sequences. The result of one such sequence is given below.



Local function assignment to regions by PFP for top 5 predictions in each GO category.



Prediction scores for top 5 predictions in each GO category GO term	Short Def	Raw Score	Term Type
GO0008544	epidermis development	31093.65	BP
GO0009405	pathogenesis	28348.88	BP
GO0007605	perception of sound	18814.05	BP
GO0032154	0032154	15815.04	BP
GO0018149	peptide cross-linking	14607.87	BP
GO0003824	catalytic activity	19268.02	MF
GO0045502	dynein binding	17966.18	MF
GO0004674	protein serine/threonine kinase activity	16382.07	MF
GO0005200	structural constituent of cytoskeleton	16321.62	MF
GO0004872	receptor activity	11752.9	MF
GO0016021	integral to membrane	16010.64	CC
GO0000228	nuclear chromosome	10695.14	CC
GO0015629	actin cytoskeleton	10389.45	CC
GO0001533	cornified envelope	10252.62	CC

Figure 5: PFP Results.

The result of all the sequences are tabulated and find weather the conserved regions of all the sequences possess same function or not.

S.NO	NCBI gi number	Function predicted	Raw score	Term type
1	7657017	epidermis development	31093.65	BP
		Pathogenesis	28348.88	BP
		perception of sound	18814.05	BP
		32154	15815.04	BP
		peptide cross-linking	14607.87	BP
		catalytic activity	19268.02	MF
		dynein binding	17966.18	MF
		protein serine/threonine kinase activity	16382.07	MF
		structural constituent of cytoskeleton	16321.62	MF
		receptor activity	11752.9	MF
		integral to membrane	16010.64	CC
		nuclear chromosome	10695.14	CC
		actin cytoskeleton	10389.45	CC
		cornified envelope	10252.62	CC
		cell surface	7997.1	CC
2	92110019	epidermis development	31093.65	BP
		Pathogenesis	28348.88	BP
		perception of sound	18814.05	BP
		32154	15815.04	BP
		peptide cross-linking	14607.87	BP
		catalytic activity	19268.02	MF
		dynein binding	17966.18	MF
		protein serine/threonine kinase activity	16382.07	MF
		structural constituent of cytoskeleton	16321.62	MF
		receptor activity	11752.9	MF
		integral to membrane	16010.64	CC
		nuclear chromosome	10695.14	CC
		actin cytoskeleton	10389.45	CC
		cornified envelope	10252.62	CC
		cell surface	7997.1	CC
3	29789405	Synaptogenesis	22741.89	BP
		protein-nucleus import	17711.21	BP
		positive regulation of I-kappaB kinase/NF-kappaB cascade	16273.19	BP
		carbohydrate metabolism	15979.02	BP
		SOS response	14951.04	BP
		Catalytic activity	43390.77	MF
		receptor activity	18639.08	MF

S.NO	NCBI gi number	Function predicted	Raw score	Term type
4	14149997	perception of sound	341455.4	BP
		Synaptogenesis	170897.63	BP
		protein transport	163190.59	BP
		plasma membrane repair	157105.91	BP
		protein-nucleus export	147918.06	BP
		protein-tyrosine kinase activity	203176.02	MF
		GTPase activity	171778.6	MF
		signal transducer activity	165762.94	MF
		protein serine/threonine kinase activity	134087.24	MF
		exonuclease activity	119295.99	MF
		actin cytoskeleton	324442.32	CC
		cornified envelope	186395.29	CC
		septin ring	150602.05	CC
		ciliary rootlet	124790.73	CC
		cell surface	102968.93	CC
5	169161100	perception of sound	341455.4	BP
		Synaptogenesis	170897.63	BP
		protein transport	163190.59	BP
		plasma membrane repair	157105.91	BP
		protein-nucleus export	147918.06	BP
		protein-tyrosine kinase activity	203176.02	MF
		GTPase activity	171778.6	MF
		signal transducer activity	165762.94	MF
		protein serine/threonine kinase activity	134087.24	MF
		exonuclease activity	119295.99	MF
		actin cytoskeleton	324442.32	CC
		cornified envelope	186395.29	CC
		septin ring	150602.05	CC
		ciliary rootlet	124790.73	CC
		cell surface	102968.93	CC
6	157266285	epidermis development	51134.59	BP
		Synaptogenesis	20540.84	BP
		protein-nucleus import	16706.75	BP
		positive regulation of I-kappaB kinase/NF-kappaB cascade	15838.39	BP
		carbohydrate metabolism	15620.98	BP
		catalytic activity	42484.21	MF
		receptor activity	17603.75	MF

S.NO	NCBI gi number	Function predicted	Raw score	Term type
7	83415174	ciliary or flagellar motility	691958.57	BP
		Neurogenesis	683191.29	BP
		regulation of embryonic development	355724.17	BP
		membrane protein ectodomain proteolysis	326061.39	BP
		perception of sound	310562.69	BP
		GTPase activity	312354.27	MF
		metallopeptidase activity	286230.15	MF
		protein-tyrosine kinase activity	285182.71	MF
		receptor activity	233443.7	MF
		protein transporter activity	213372.14	MF
		Midbody	341319.99	CC
		septin ring	330489.87	CC
		growth cone	275600.06	CC
		Synaptosome	200571.35	CC
		ciliary rootlet	187282.01	CC
8	119709825	Pathogenesis	54825.47	BP
		RNA splicing	42467.13	BP
		Neurogenesis	27276.7	BP
		ion transport	21731.84	BP
		Spermatogenesis	20643.32	BP
		GTPase activity	49460.02	MF
		protein heterodimerization activity	25501.28	MF
		protein-tyrosine kinase activity	24800.97	MF
		structural molecule activity	20357.76	MF
		hydrolase activity	18802.54	MF
		Myosin	29385.68	CC
		septin ring	26512.47	CC
		alpha-glucosidase complex	24887.99	CC
		growth cone	18243.4	CC
		ciliary rootlet	18141.28	CC
9	156071420	Pathogenesis	52781.82	BP
		RNA splicing	39914.09	BP
		Neurogenesis	26114.97	BP
		positive regulation of I-kappaB kinase/NF-kappaB cascade	23577.9	BP
		ion transport	20794.78	BP
		GTPase activity	47120.8	MF
		protein heterodimerization activity	24498.88	MF

S.NO	NCBI gi number	Function predicted	Raw score	Term type
10	114158664	Pathogenesis	2306360.89	BP
		protein transport	416503.48	BP
		polysaccharide catabolism	203038.47	BP
		glycerol-3-phosphate catabolism	130474.59	BP
		brain development	106689.33	BP
		metal ion binding	607029.88	MF
		ATPase activity	463755.4	MF
		metallopeptidase activity	297063.2	MF
		GTP binding	252123.28	MF
		zinc ion binding	225511.21	MF
		cell surface	658803.27	CC
		Chromosome	356615.29	CC
		actin cytoskeleton	143564	CC
		Myosin	74258.4	CC
		cornified envelope	66980.2	CC

Table 1: PFP Results for the 10 Consensus Sequence.

From the results of Protein Prediction server, it is found that those consensus sequences possess the similar function **pathogenesis**, so it might be considered as a domain.

6.4) The identified domain is submitted to the secondary structure prediction tool and the results are given below:

TOOL	Alpha helix %	Extended strand %	Beta turn %	Random coil %
SOPM	52.67	10.67	4.67	32.00
SOPMA	56.00	8.67	6.00	29.33
GOR IV	46.67	11.33	0.00	42.00
SIMPA96	56.95	7.95	0.00	34.44

Table 2: Secondary Structure Prediction Results.

Thus the secondary structure of the domain is predicted and it is found that it will contain majority of helices and random coils.

7. CONCLUSION:

The hypothetical proteins from the Human Chromosome 1 are retrieved and the sequences are clustered based on sequence similarity. A set of ten sequences are found to be more similar and they contain consensus sequence. These sequences are fortunately having the similar function, all because of the presence of that consensus sequence.

Thus the consensus sequence might be the novel domain for those ten sequences of Human Chromosome 1 which is not yet identified. The novel domain involved in the pathogenesis functionality. The secondary structure of the identified domain contains majority of the helix and random coil.

Thus this project may provide the pave to identify the novel domains which are not yet identified. This project is just an assumption and it is very difficult to cluster the large sets of hypothetical sequence at a time and even though the identified common domain possesses identical residues more, it shows few similarity residues.

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