Background and Research Scheme IPRO 318 Searching for novel drug targets

Introduction

Over the course of the term, IPRO 318 made progress in the "[Search] for Novel Drug Targets." This IPRO is part of an ongoing effort to try to find potential drug targets on pathogenic proteins. During past terms, IPRO 318 has compiled information on proteins that are known to be involved in diseases in a disease gene database. By comparing this to a database of hypothetical genes, possible high value proteins were found. This term IPRO 318 continued the lab work on the high value proteins found in previous semesters, found new ones and began lab work on those proteins, continued work on the databases, and started a new allergen database.

Background

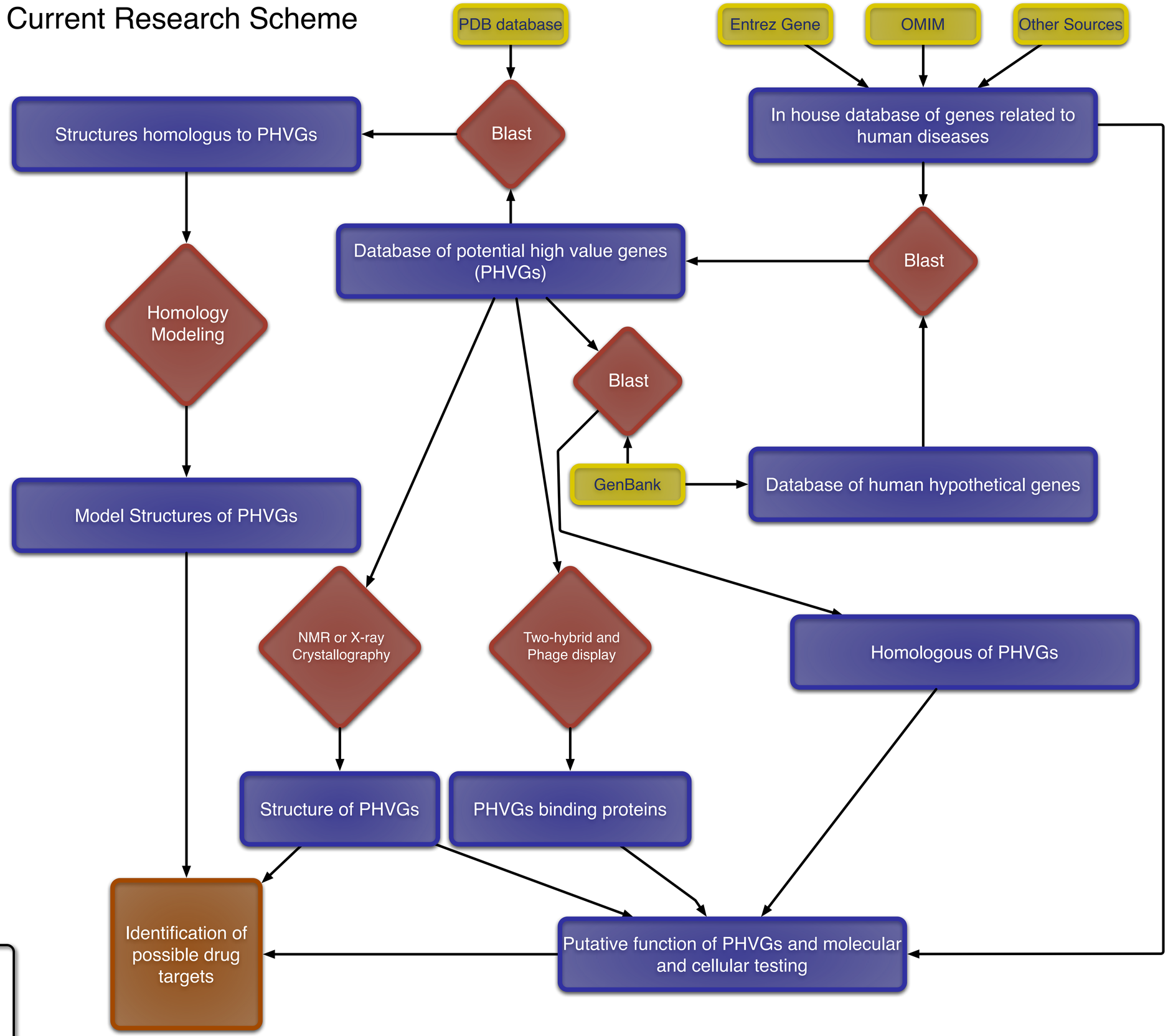
The majority of the gene functions in the human genome (see ref.1 for the first draft) are still unknown. Many of those genes may be a valuable drug target for numerous human health issues. The discovery of novel molecular drug targets is becoming more efficient with the use of powerful high throughput technologies, gene expression microarrays, and proteomics methods (2,3). However, presently these targets comprise of only a small fraction of the human genome and determining the potential pharmaceutical applications of genes and their encoded proteins is still the rate-limiting step in deriving value from this gene sequence information. Thus, it is important to identify new drug targets in the post genomic era.

The final goal of this IPRO is to find novel drug targets. In this process the first objective that must be met is the creation of three databases. The first database, which contained hypothetical gene products predicted from the human genome, was almost completed during the Spring of 2007. The second database contains proteins that are known to be involved in diseases. The final database contains potential drug targets based on sequence alignment between the first two databases. Our team also started work on a forth database, an allergen database. Our next objective this term was to continue research on one of the proteins found last term and to find its interaction with Cry AB in yeast. Our final objective was to find more high value proteins and begin work on determining their roles in the cells. Hopefully future IPRO 318s will continue to work on the databases, do research on the high value proteins found, and find more high value proteins.

Searching For Nobel Drug Targets

Illinois Institute of Technology

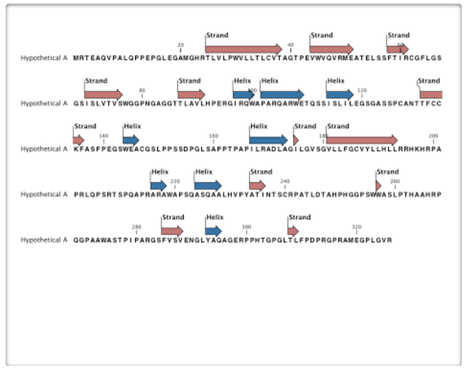
Current Research Scheme



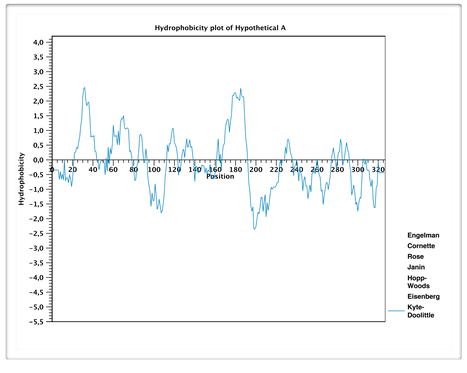
Methods and Research diagrams IPRO 318 Searching for novel drug targets

Computer Analysis and Website Team Methods

The computer analysis and web design team's main objectives for the semester included blasting the current databases produced by previous semesters IPROs. The results from this blast were then screened for well qualified genes. Well qualified in this case indicated that they were globular, and had a good mix of hydrophobic and hydrophilic regions. This was important as globular proteins are more likely to carry drug targets in them. Other than the website, the other main task of this group was to build up a new allergen database for future IPROs. This process involved querying established allergen databases and then linking these results with the public genome databases to create a custom in-house database which will be blasted with the hypothetical gene database in semesters to come.



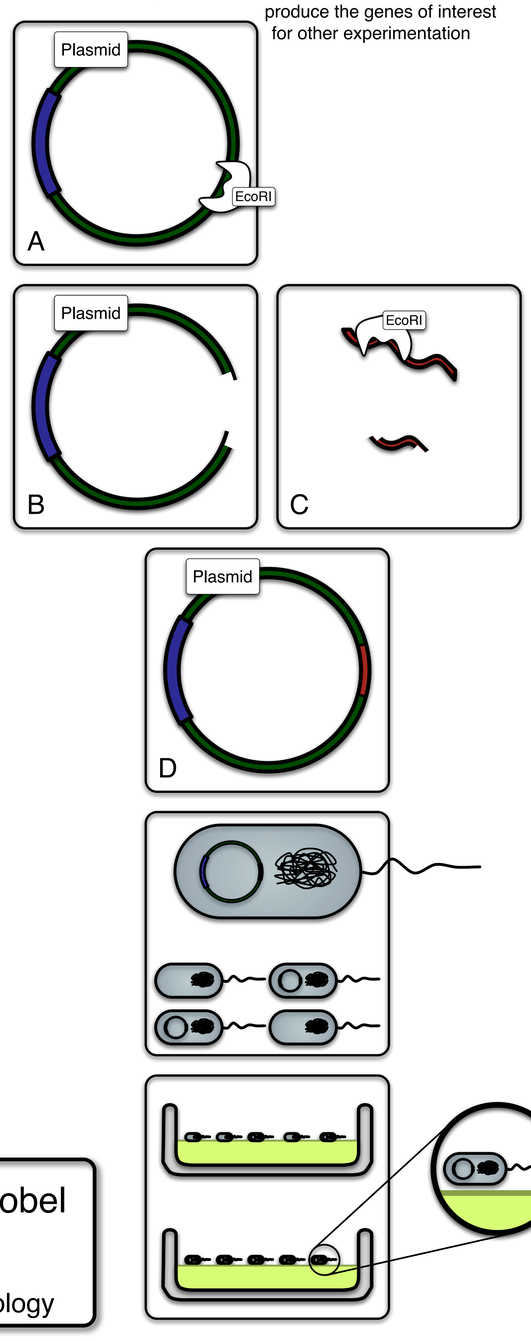
Structural components of hypothetical gene 1
Red components Alpha Helixes
Blue components Beta Sheets



Hydrophobicity of hypothetical gene 1

Figure of Bacteria Transformation:

(A) Using a restriction enzyme a bacterial or yeast plasmid is cut to allow insertion of our gene of interest
(B) The plasmid carries an antibacterial resistance gene (blue section on the diagram)
(C) the Gene of interest is cut with the same restriction enzyme to allow insertion into the plasmid
(D) the gene of interest of is placed into cut plasmid
(E) the plasmid is transformed into bacterial or yeast cells and cloned to propagate the plasmid
(F) Transformed bacteria are plated on anti-biotic growth medium and only bacteria with transformed plasmids grow on this medium, the bacteria and yeast are promoted to produce the genes of interest for other experimentation



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New Gene Group Methods

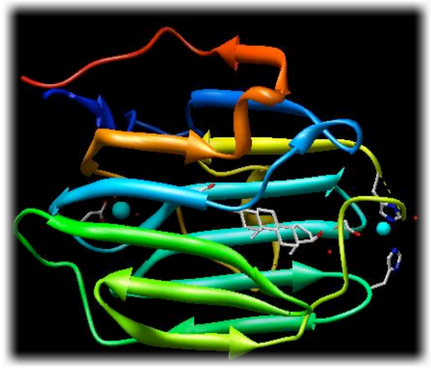
Using an existing database compiled from the previous Spring IPRO318, hypothetical gene sequences of various illnesses were analyzed. From a list of thousands, several sequences were subjected to the BLAST algorithm to determine their homology with known illnesses. Two sequences were chosen due to their high homology in addition to their globular characteristics. These were ordered from a commercial source in order to find potential novel drug targets through experimentation. Molecular biological principles, namely Polymerase Chain Reaction (PCR), were exploited in order to amplify the sequence. Once amplified, the sequence was converted to a plasmid for insertion into a yeast cell, which was induced to produce the protein coded by the insert. This protein can then be purified and concentrated. This can be used to study the structure-function relationship. We can also use protein expression to study the protein-protein interaction. The team members will extract from vectors using affinity columns and various electrophoresis separation techniques. Finally, the extracted gene will be spliced into custom plasmids, and it will be run by two-hybrid system.

Continuation Group Methods

Our group has created two mutations of Cry-AB to observe whether these mutations effectively prohibit Cry-AB interaction with the H1 gene in a yeast two-hybrid system using a reporter plasmid. The wild type (normal gene) Cry-AB as well as both mutant strains were put into the plamid vectors, 4-5. In order to test the Cry-AB/H1 interaction the H1 gene was put into the 202 vector. These plasmids were then transformed into yeast to test the observe the interaction, or possible lack there of, between the wild type, mutant strains, and H1. If the transcribed and translated proteins of Cry-AB and H1 are interacting, they will form a DNA binding-activator complex that will in turn transcribe and translate an indicative (usually colored) protein. By testing the wild type of Cry-AB we continue to confirm the interaction between the non-mutant strain of Cry-AB and hypothetical gene H1. We will then be able to test the interaction between the mutant strains of Cry-AB 590 and 600 and H1. The empty 202 vector, meaning H1 was not transformed into it, is used as a control to assure that it is indeed H1 Cry-AB is interacting with and not part of the carrier vector.

Computer Analysis and Website Team Results

A website has been finished. The website currently displays most of our research information, our goal and our progress. The website also contains a gallery of homolog protein 3D structures of our hypothetical genes. Aside from that, the website has a guest book service as well as a shout box for people to communicate short bursts of information in real time. There are molecular biology protocols on the website that we currently use or will use in the future. The allergen database has been mostly done. The protein blast database has been done too and have been used for the search of high potential value genes.



Above: Model of new Gene
Above right: Yeast Growth of continuation group results
Right: Gel picture of CRY-AB mutagens 600 and 590 a DNA Marker and the hypothetical gene in lanes from left to right respectively
Below right: Growth plates of Continuation group's yeast

Continuation Group Results

At the end of the previous team's term, there was interaction discovered between the hypothetical H1 gene product and the proteins related to heart disease and cataracts known as Crystallin AB or Cry-AB. Through literature, two separate mutations of the Cry-AB gene were known to exist. The mutations were then reproduced in the lab in order to test if Cry-AB mutations would disrupt its interaction with the H1 gene. Through PCR recombination, the mutation P205 was induced and placed into T2 bacterial vectors. Once replicated, the bacterial vectors were transformed into yeast cells in which interaction could be tested. Therefore, with the mutations induced and vectors for yeast prepared the next logical step would be to proceed with experimentation to test for interactions.

New Gene Group Results

The selection of viable proteins was completed early in the course. There were several proteins that did not match our criteria of being viable (globular, small, similar to human protein, etc). We narrowed down the selection to three genes that would be viable targets for research. The new gene group successfully made it to the lab and began to amplify the gene through PCR. Two of the three genes selected have been replicated. The third gene had trouble showing up on the gels and therefore was unable to be advanced further.