ILLINOIS INSTITUTE OF TECHNOLOGY

Transforming Lives. Inventing the Future.

IPRO 318: Searching for Novel Drug Targets

INTRODUCTION TO IPRO 318

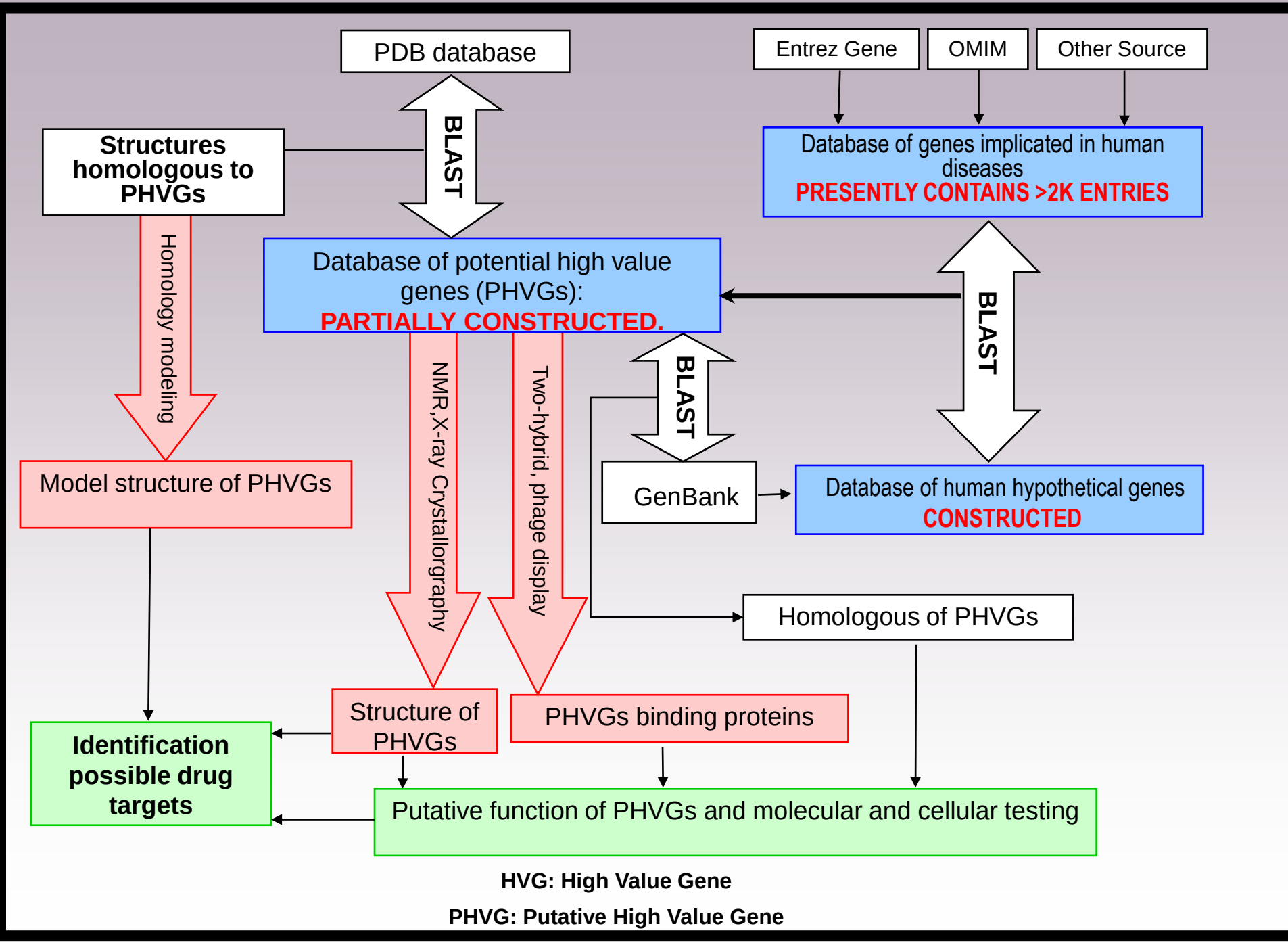
Over the last century, there has been a major shift in human diseases. Once the major cause of human fatalities was infectious diseases, but today we are dealing with lifestyle and genetically linked diseases. Effective drug development today is based heavily on the structure-function relationship as well as protein-protein interaction. Our goal in this IPRO is to identify new drug targets among proteins with unknown functions.

METHODS FOR TARGET IDENTIFICATION

Using three databases constructed by IPRO 318

- Database 1- gene-disease relationships
- Database 2- hypothetical genes
- Database 3- combination database of 1 and 2 of hypothetical drug targets

http://snx2.biol.iit.edu/ipro318/


PDB database
Entrez Gene
OMIM
Other Source
BLAST
Structures homologous to PHVGs
Database of genes implicated in human diseases
PRESENTLY CONTAINS >2K ENTRIES
Homology modeling
Database of potential high value genes (PHVGs):
PARTIALLY CONSTRUCTED.
BLAST
BLAST
NMR,X-ray Crystallorgraphy
Two-hybrid, phage display
Model structure of PHVGs
GenBank
Database of human hypothetical genes
CONSTRUCTED
Homologous of PHVGs
Structure of PHVGs
PHVGs binding proteins
Identification possible drug targets
Putative function of PHVGs and molecular and cellular testing
HVG: High Value Gene
PHVG: Putative High Value Gene

WHAT TO DO ONCE HYPOTHETICAL TARGETS ARE IDENTIFIED?

2 Routes for Novel Drug Targets

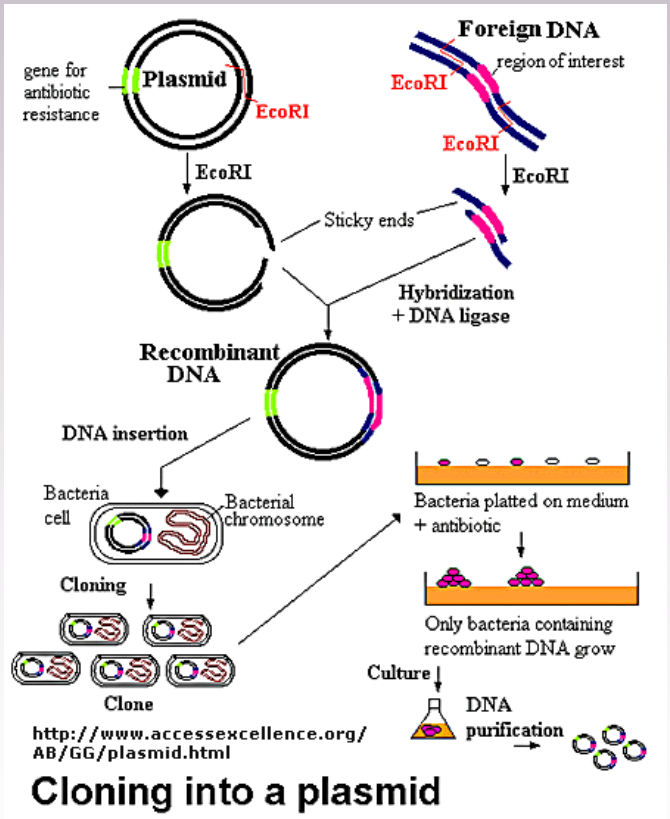
- Route 1- The properties of putative high values gene can be studied by molecular modeling, protein expression, as well as determining the structure of the protein product of the gene.

- Route 2- Identifying interactions among proteins for putative high value genes by two-hybrid screens in yeast.

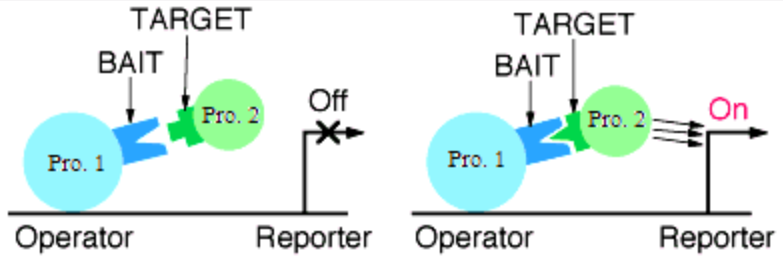
Thus far, we have been focused on four hypothetical genes. The product of these four genes are named H1, H2, H3, and H4 (also known as KIAA).

STUDYING HYPOTHETICAL TARGETS

Vector Insertion



Once the plasmid has been inserted into a cell, it can be 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, as shown below.



RESULTS

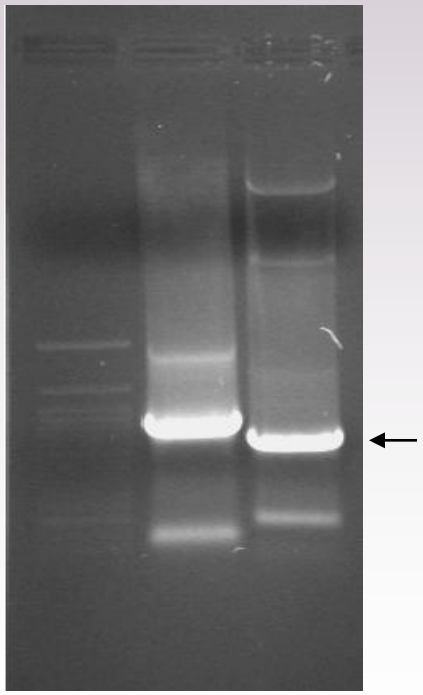
- Database Expansion to over 2000 disease and their respective protein and gene number
- Successful cloning of the four hypothetical genes (H1, H2, H3 and H4) into vectors
- Successful over-expression of H1 in bacteria
- Interactor identification for the genes

RESULTS (CON'T)

Cloning of H1

318-H1 PCR1

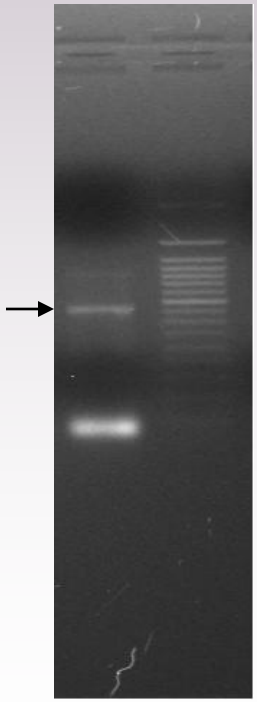
H1



Sept. 21 2006

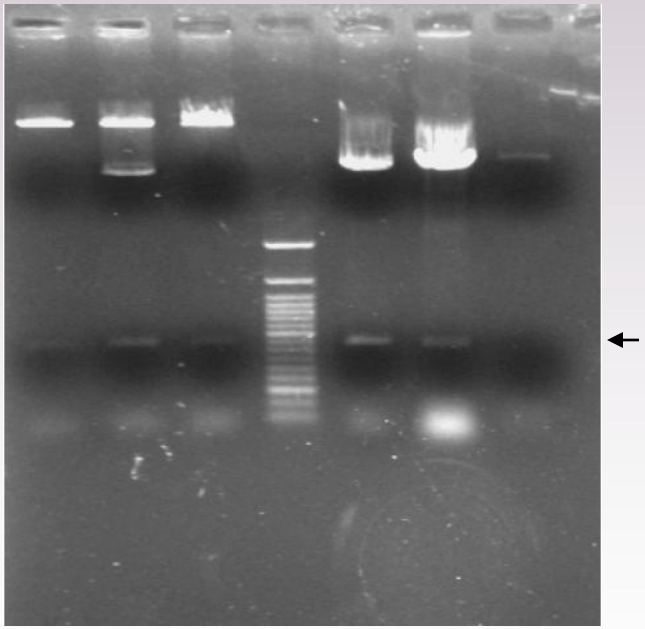
318-H1 PCR2

H1



Sept. 26 2006

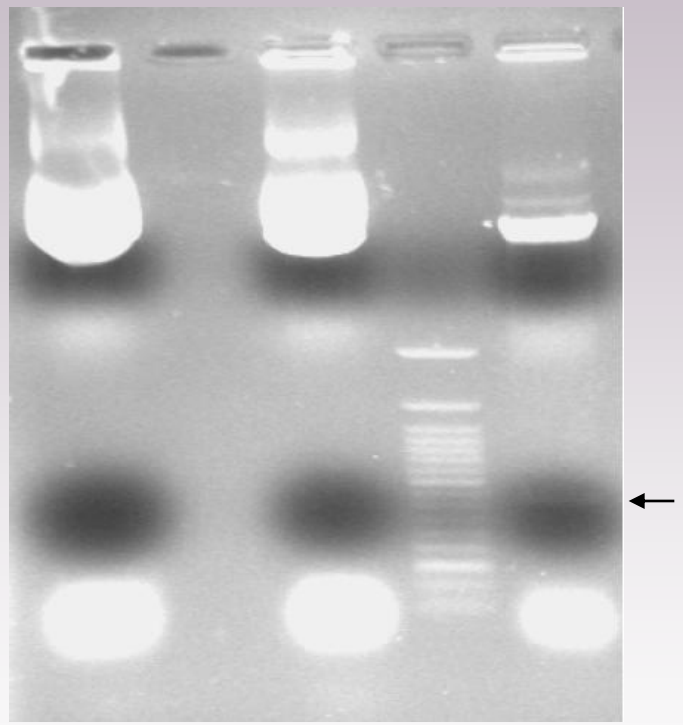
pEZY202-318H1



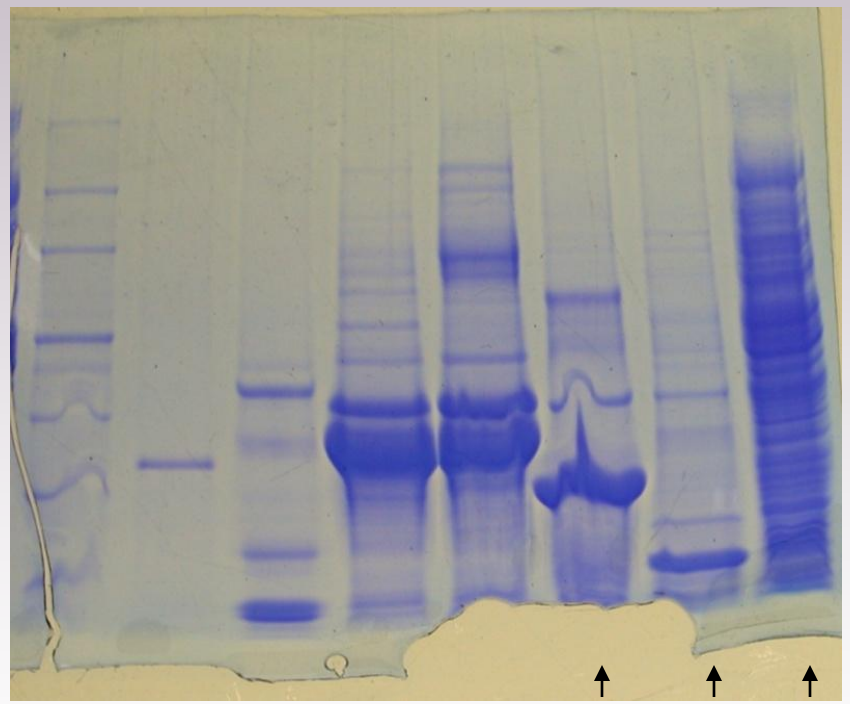
Oct. 9 2006

RESULTS (CON'T)

pRSF-318-H1 miniprep



H1 Induction



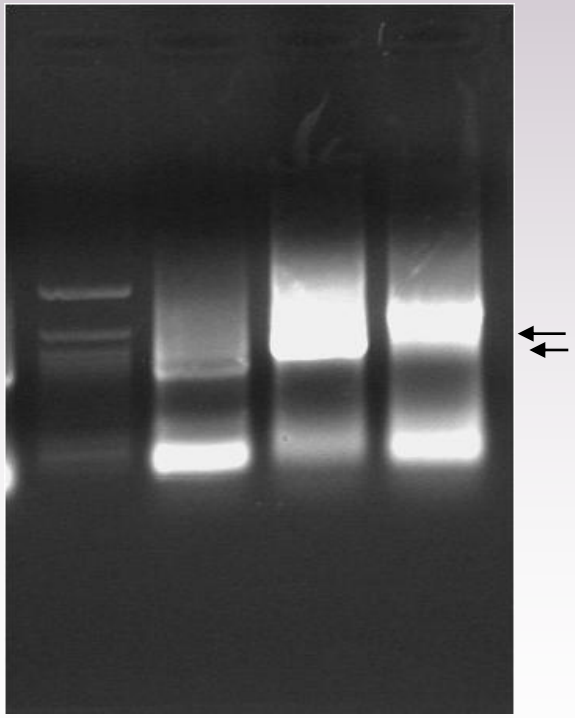
M M sumo-H1 H1IB H1 supernant

RESULTS (CON'T)

Cloning of H2 and H3

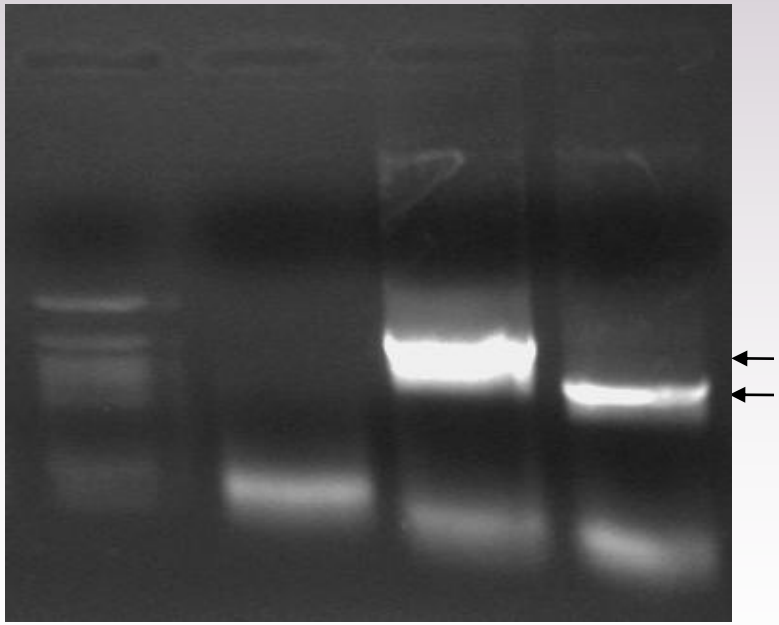
318H2 H3 PCR2

Marker H2 H3



318-H2 H3 PCR1

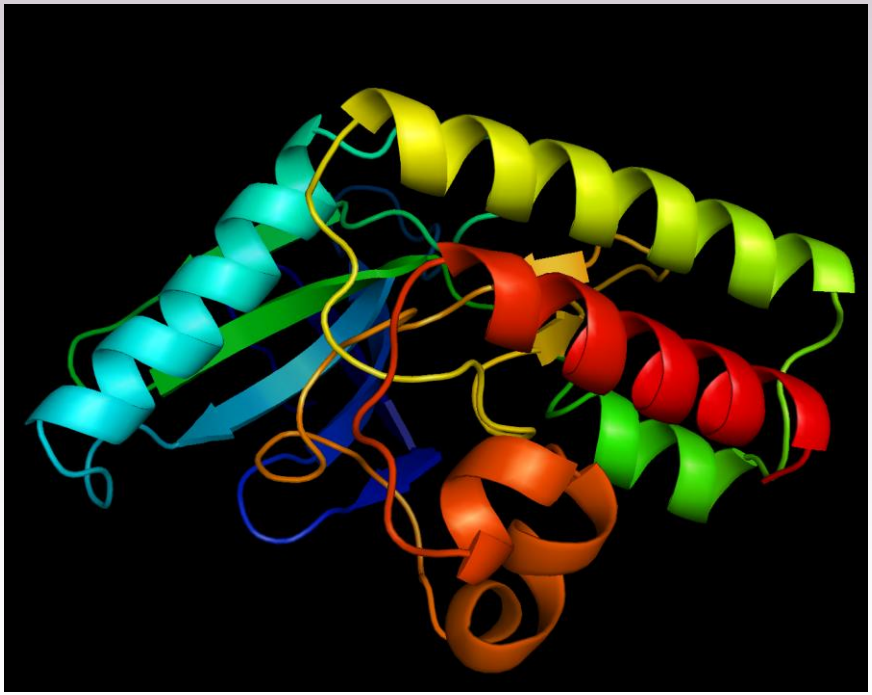
Marker 318H3 318H2



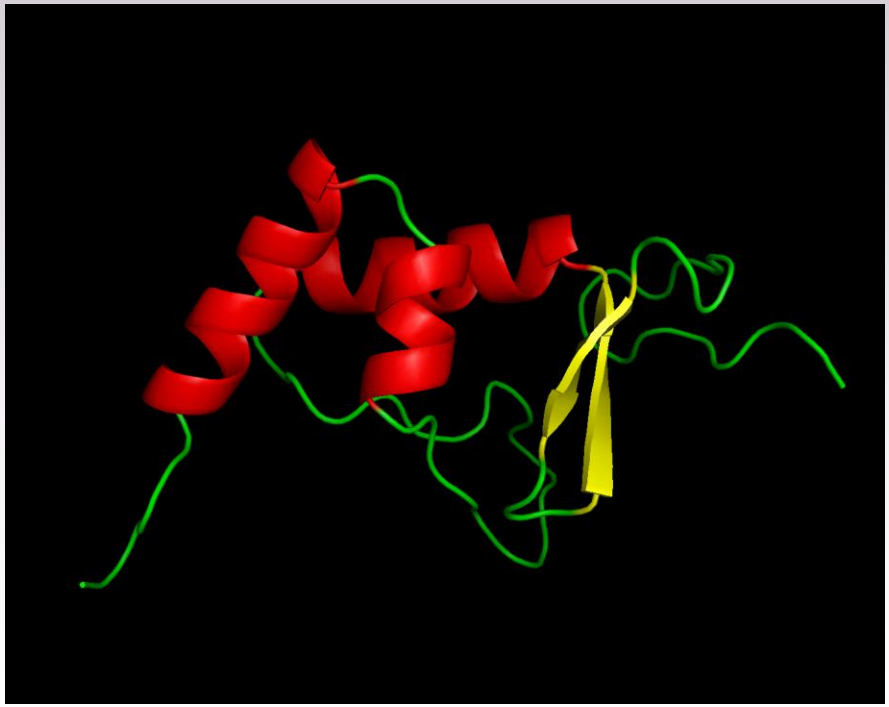
RESULTS (CON'T)

Homology Model of H2 and H3

H2 Model



H3 Model

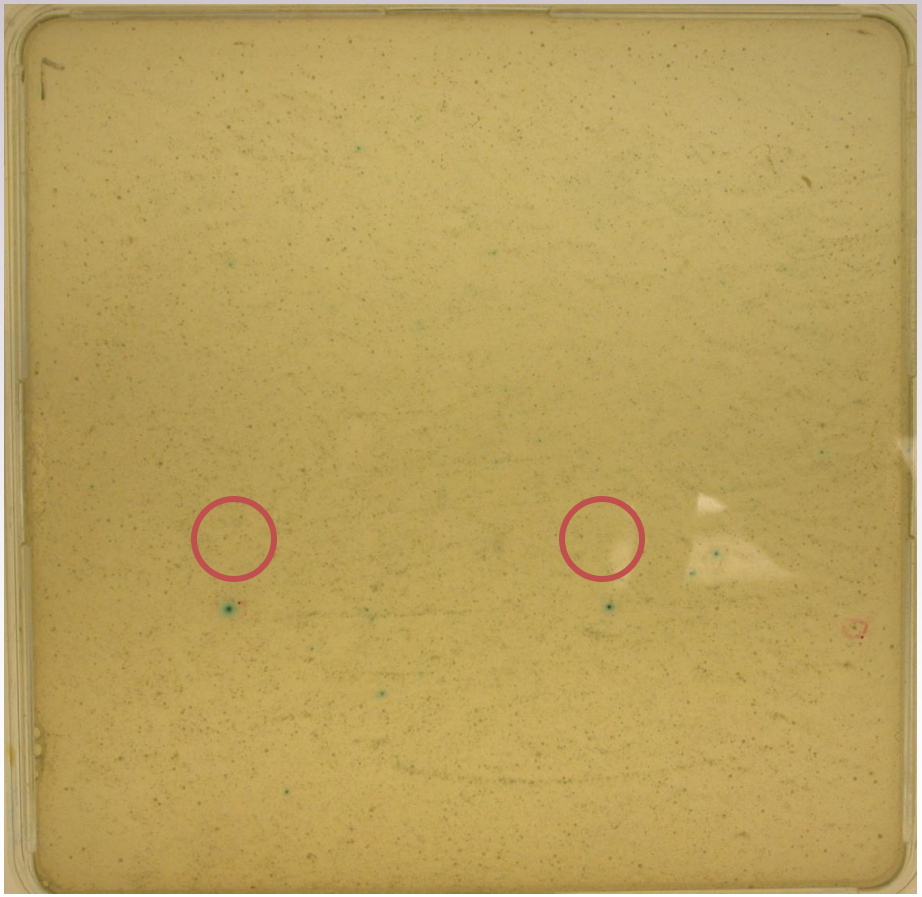


RESULTS (CON'T)

318 H1 UHWL/X-Gal Plate

- Two-Hybrid Screen for (318H1) Interacting Protein

- Blue Colonies for UHXL on Dropout Plates Indicating Putative Interactors



FUTURE OF IPRO 318

- Completion of the Database
    - Expansion of disease list
    - Inclusion of related protein and gene sequences
    - Search engine for easier use
- Further research into novel drug targets
    - DNA microarray
    - Proteomic techniques

IPRO 318 TEAM

Advisor
- Dr. Yu zhu Zhang

Website Designer
- Martina Dolejs

Database
- Hyunsuk Kim
- Calvin Wu
- Lindsey Polich

Protein Expression
- Amit Kamdar
- Tengchaun Jin
- Ronak Desai

Yeast Two-Hybrid
- Joshua Marell
- Josh Knox
- Floriann H. Stankovich
- Vrudhdhi Patel









- H1: similar to Beta-glucuronidase which is implicated in diabetes
- H2: similar to inositol polyphosphate-5-phosphatase which is implicated in diabetes
- H3: Similar to androgen receptor associated protein 54, implicated in Kennedy's disease
- H4: similar to PHF11 which is coded by the asthma susceptibility gene