2011 Professional Development Report
Tsunemi Yamashita, Associate professor of Biological Sciences

Project Title: High throughput expression cloning studies for proteomics

B. Abstract/ Purposes and Objectives

The development of systems to artificially create a diverse protein array from a variety of genes is an important goal in the modern world of large scale genetic studies (genomics) and cellular protein studies (proteomics). Due to the cataloging and characterization of a multitude of genes through genomic research, methods to effectively produce the proteins associated with these genes are important to further study the complex interactions proteins are engaged during cellular activity. This project will provide materials to create a protein expression system that can effectively create cultured proteins from a wide variety of genes. I plan to target two primary protein types with this work: proteins associated with scorpion toxins (Valdez-Cruz et al. 2004) and those from the Anosmin 1 gene family.

C. Brief review of the Research procedure

From the advent of modern genomic and proteomics research, new methodologies have been developed to quickly produce hundreds of proteins from many genes identified through genomic analyses. One of the new methods, Ligation Independent Cloning (LIC), is considered a robust alternative to standard methods to generate a wide variety of proteins from isolated genes. LIC has gained popularity due the reduced cost and simplicity (SGC Structural Genomics Consortium- 2008, Eschenfeldt et al. 2009). As this method is routinely used in 9 of 14 Structural Genomics (SG) centers, we are encouraged that it can be a viable alternative to generate protein expression clones (SGC 2008). There are two main components to this project:

1. The creation of DNA with the gene of interest and DNA insertion into bacterial host cells. Here I plan to isolate and remove the gene for the proteins with molecular techniques and place them into a circular piece of plasmid DNA for storage and then place the DNA into a bacterial cell for subsequent protein production. More specifically, the LIC primers will contain the appropriate sequences for T4 DNA polymerase digestion. After PCR amplification of the targeted insert regions, the gene insert and prepared vector will be annealed and then transformed into bacterial host cells. To verify transformed bacteria with the appropriate gene insert, we will isolate plasmid from transformed cells, amplify with PCR primers targeted to the gene insert and visualize products with agarose gel electrophoresis. In addition, purified plasmids will be sequenced for additional verification and insert sequence fidelity.

2. Initial small scale protein production

After conformation of a robust plasmid with the gene of interest, the bacterial cells will be grown under conditions for protein expression. An initial check of protein production will occur with SDS gel electrophoresis of cellular extracts after expression induction. These gels can discriminate among many different proteins and allow the identification of expected protein production. If protein expression is seen with an appropriate size band to the targeted protein on the SDS gel, larger cultures will be created for large scale protein production and purification.

D. Summary of Findings

I was able to complete all the work needed to create a plasmid with the sodium toxin gene inserted into it. I was not able to complete the second component as I began my sabbatical in Japan. However, this work has given me the ability to complete similar research in Japan during my sabbatical, and I plan to complete this work with an undergraduate student during the 2012 spring semester. I also plan to modify lab exercises in my Molecular Genetics class to incorporate several of the techniques learned through this experience.

E. Conclusions

The LIC cloning method proved to be a simple and straight forward method of gene isolation and creation of bacteria capable to artificially produce proteins. This project has significantly improved my capabilities for sabbatical research and provides a new avenue of research methods for my work and will assist in updating class lab exercises. I am appreciative of the funding and the administrative support for this project.

F. Bibliography

Eschenfeldt WH, Lucy S, Millard CS, Joachimiak A, and Mark ID. 2009. A family of LIC vectors for high-throughput cloning and purification of proteins. Methods Mol BioI. 498: 105-15.

Structural Genomics Consortium1,2,3, Architecture et Fonction des Macromolecules Biologiques4, Berkeley Structural Genomics Center5, China Structural Genomics Consortium6,7, Inte~rated Center for Structure and Function Innovation8, Israel Structural Proteomics Center, Joint Center for Structural Genomics10,11, Midwest Center for Structural Genomics12, New York Structural GenomiX Research Center for Structural Genomics13,14,15,16,17, Northeast Structural Genomics Consortium18,19, Oxford Protein Production Facility20, Protein Sample Production Facility, Max Delbruck Center for Molecular Medicine21 , RIKEN Structural Genomics/Proteomics Initiative22, and SPINE2-Complexes23,25
2008. Protein production and purification. *Nature Methods* 5: 135 -146.

Valdez-Cruz, N.A., Davila, S., Licea, A., Corona, M., Zamudio, F.Z., Garcia-Valdes, J., Boyer, L. & Possani, L.D. (2004) Biochemical, genetic, and physiological characterization of venom components from two species of scorpions: *Centruroides exilicauda* Wood and *Centruroides sculpturatus* Ewing. Biochime, 86, 387-396.