SUMMER RESEARCH OPPORTUNITY

IN

MOLECULAR BIOSCIENCES

AT

UNIVERSITY OF PUERTO RICO-RIO PIEDRAS

Research Experience for Undergraduates (REU) Sites award has been made to the University of Puerto Rico-Rio Piedras that will provide training for 10 students for ten weeks, during the summers of 2012-2014. The program focuses on molecular biosciences -- the application of molecular biology and bioinformatics to major areas of biology like molecular genetics, microbiology, neuroscience and evolution. The Department of Biology faculty will serve as mentors, who will offer a wide range of interesting and exciting projects for the students. Parallel to the research work, students will participate in a series of workshops and seminars, such as the responsible conduct in research, professional communication skills, career opportunities, and the graduate school application process. The REU students have access to a rich array of individual mentor's facilities as well as many interdepartmental labs and centers with core facilities such as electron and confocal microscopy, sequencing, microarray analysis, proteomic analysis, etc. In addition, students will write a scientific report on their research project and present their work at an end-of-program symposium. The program's multi-phase recruitment effort consists of both traditional "hard copy" formats as well as digital-based advertising. Students are selected based on academic record, research performance, and potential for outstanding research in biomolecular sciences. Students are tracked to determine their continued interest in their academic field of study, their career paths, and the lasting influences of the research experience. Information about the program will be assessed by various means, including use of an REU common assessment tool. More information is available by visiting http://biology.uprrp.edu/undergraduate/ur_research_oportunities.htm, or by contacting the PI (Dr. Migdalisel Colon at lisycolonberlingeri@yahoo.com) or the co-PI (Dr. Jose E Garcia at jegarcia@hpcf.upr.edu).

Students choose from the following research projects:

Baerga, Abel- Biosynthesis of natural products

Dr. Baerga's laboratory is focused on the biosynthesis of natural products in bacteria from different environments. One of the main projects in the lab is aimed at detecting genes for the biosynthesis of natural products in human intestinal bacteria. Strains of commensal intestinal bacteria have been found to contain genes for the biosynthesis of polyketide toxins. Furthermore, many beneficial compounds with estrogen-like activity are made by bacteria of the human gut microflora. They are currently delineating the geographic distribution of these genes in the Puerto Rican population and their possible correlation with environmental factors.

Bayman, Paul- ***Interactions Between Fungi and Plant and Animal Hosts***

Research in Dr. Bayman's lab focuses on interactions between microorganisms (principally fungi) and other organisms. Current projects include studies on host-pathogen relations in diseases of marine invertebrates, specificity of orchids for mycorrhizal fungi, phylogeography of opportunistic pathogens, and biocontrol. Potential projects for summer students include: testing mutants of *Aspergillus* for virulence using *Drosophila,* relationship of substrate and mycotoxin production in Aspergillus, and pathogenicity of potential biocontrol fungi to insects. The REU student will help in the identification of fungi samples using molecular techniques.

Behra, Martine- Regeneration of fish lateral line

Using the zebrafish embryo, Dr. Behra's laboratory is trying to unravel the genetics of regeneration in a sensory neural tissue, which is called the lateral line. This organ is thought to be the evolutionary precursor of sensory epithelia of the inner ear in higher vertebrates. The lack of regeneration of these tissues in mammals is the leading causes for human deafness. Conducting a parallel approach of *in toto* live imaging and transcriptome analysis of the different cell types, researchers hope to gain a fundamental understanding of the process in a sensory tissue , which will then be extrapolated to general principles of regeneration. A summer student will be involve in the characterization of various genes expressed in the lateral line cell population.

Cadilla, Carmen- Gene Regulation by the Transcription Factor TWIST2

Dr. Cadilla and her colleagues study the basic helix-loop-helix (bHLH) transcription factor TWIST2 gene. The expression pattern of TWIST2 mRNA during mammalian development suggests that it functions as a regulator of gene expression in a subset of mesenchymal cell lineages including developing dermis. Gene expression profiles of fibroblasts harboring a TWIST2 mutation and normal controls were examined by microarray analysis. Genes found to be differentially regulated included various genes involved in the inflammatory response, TGFβ signaling, and development. Work in the lab is presently focused in elucidating the mechanisms of TWIST2 gene regulation, using human skin fibroblasts and other cell mammalian cell lines. Summer students will engage in gene regulation studies that will help identify cis elements used in direct action of Twist2 using a variety of techniques, such as DNA binding techniques (gel shift and ChIP assays) and transfection of expression constructs into mammalian cells, as well as indirect mechanisms that involve protein-protein interactions and chromatin structural and chemical changes (epigenetics).

Dominguez-Bello, Maria G- ***Bacterial Characterization of the Microbiome***

The research in Dr. Dominguez-Bello's laboratory integrates data from microbiology, genomics/metagenomics, ecology, physiology and anthropology and biostatistics to address broad questions of how microbes and hosts interact. One focus is on how these interactions drive microbial diversity, and which aspects and circumstances define pathogen and commensal microbiota. They study diversity of the bacterial microbiota and the symbiosis in vertebrates, in particular, the symbiosis in the foregut of mammals and birds, effect of lifestyle on the microbiota of animals and humans, developmental aspects of the human and mice microbiota, antibiotic resistance in the microbiota, and bacterial adaptations to their hosts in a system of long-term colonization such as *Helicobacter pylori* in humans. Technical aspects of the research involve DNA amplification, phylogenetic analysis, pyrosequencing and metagenomics. The student will be involved in summer activities concerning the processing of digestive samples from wild animals (green turtle and gorillas) to hybridize in DNA microarrays.

García-Arrarás, Jose E.- ***Gene Profiling of Regeneration Processes***

Dr. Garcia-Arrarás has pioneered the use of the echinoderm *Holothuria glaberrima* to study the process of regeneration and organogenesis. His research focuses on the molecular aspects of regeneration, specifically on the genes that are important for the regeneration process to occur. His lab has generated an expressed sequence tag (EST) database for *H. glaberrima* sequences obtained from three cDNA libraries, one made from non-eviscerated (normal) intestinal tissue, and two from regenerating (3-days and 5-7-days after evisceration). They have identified and characterized several genes involved in holothurian intestinal regeneration using microarrays and other techniques. Their work is aimed at finding different profiles of gene expression and at determining the function of specific genes during the process of regeneration. Summer students will be involved in bioinformatics analyses to determine gene sequences, structural domains and gene characterization. In addition, the evolution of particular genes will be targeted.

Giray, Tugrul- ***Mechanisms of Plasticity in Honeybee Social Behavior***

Dr. Giray's lab uses classic and new learning assays to study honeybee social behavior, both in the field and in the laboratory. With these, they probe the neural and molecular bases of plasticity in a mini brain. Simple questions, such as if colony conditions influence behavioral development as measured by flight muscle protein expression are important within this research program and has been answered by undergraduate research students. More recently, the lab has focused on the expression of candidate genes involved in aversive learning in the honeybee brain combining behavioral, bioinformatics, and molecular techniques. This combination of experimentally accessible social behavior and techniques to probe underlying molecular mechanisms make this research program especially suitable for undergraduate research experience. Summer students will test hypotheses on molecular correlates of learning and memory in the honeybee using techniques to measure gene and protein expression in response to experimental manipulations.

Gonzalez, Carlos I.-Post-transcriptional control of Interleukin-3

Interleukin-3 (IL-3) is a pleiotropic cytokine involved in the host responses to inflammatory, immunologic and infectious stimuli. IL-3 expression is restricted to T-lymphocytes and is produced after stimulation with antigens, mitogens and phorbol esters. The regulation of mRNA decay and translation of the IL-3 transcript depends on the presence of an intact Adenosine/Uridine-rich element (ARE) in its 3'-untranslated region (3'-UTR). AREs are RNA regulatory motifs of 50-100 nucleotides that have an effect on mRNA stability and/or translation. The regulatory effects of AREs are often mediated by specific ARE-binding proteins (ARE-BPs). Recent evidence from our laboratory suggests that the conserved IL-3 ARE can reduce translational efficiency in vivo. Also, the IL-3 ARE is required to increase IL-3 protein levels following T-cell activation. UV crosslinking assays identified five ARE-BP complexes from approximately 30 to 90 kDa. Gel retardation experiments further identified the ARE-BP HuR as a factor that recognizes the IL-3 mRNA. RNA affinity coupled with

Western blot analysis and RT-PCR confirmed the presence of HuR as a component of the IL-3 ARE-BP complex. Moreover, HuR over-expression down-regulates a luciferase/IL-3 3`UTR chimeric reporter mRNA. Bioinformatics analysis using TargetScan suggests that the microRNAs miR-15a and miR-16 recognize sequences within the IL-3 3'-UTR. The main goal of this project is to characterize the role of the ARE-mediated pathway in the regulation of IL-3. Specifically, we intend to: 1) identify and characterize the minimal ARE cluster required for the regulation of IL-3 expression; 2) elucidate the regulatory function of HuR in IL-3 expression; and 3) characterize the role of miR15a and miR-16 in the post-transcriptional control of IL-3. The REU-CRIB student will use a combination of bioinformatics, molecular and biochemical approaches to better understand the roles of the ARE-mediated pathway and the microRNA-dependent regulatory network in the post-transcriptional control of IL-3.

Hrbek, Tomas- ***Quantitative and Population Genetics***

Dr. Hrbek's lab research strives to understand the relative role of adaptive and non-adaptive processes responsible for the generation and maintenance of observed patterns of biological diversity. In order to accomplish these goals, they focus on three main areas of research: 1) the use of phylogenetic and phylogeographic approaches to generate hypotheses of relationships among species and populations at different levels of hierarchical organization; 2) the use of phylogenetic hypotheses to test processes potentially responsible for differentiation of populations and species at different stages of diversification, and for the reconstruction of most likely scenarios of the evolution of complex life histories and novel structures; and 3) use of the coalescent theory to test hypotheses of past demographic events and evolutionary history. The common theme in these studies is to investigate the effect of past climate changes on genetic diversity and the distribution of genetic diversity of the study organisms.

Maldonado-Vlaar, Carmen- ***Neurobiology of Addiction***

Dr. Maldonado-Vlaar laboratory focuses on the molecular basis of addictive behaviors. Specifically her lab studies the changes in gene expression within the nucleus accumbens (NAc) that take place during cocaine dependence development. Specific genes involved in cocaine conditioning were identified using high throughput gene expression profile analysis of the NAc, during different stages of the environment-elicited cocaine conditioning. The differential expression of certain genes has been validated using PCR and other molecular biology techniques. Summer students in her laboratory will analyze the gene expression parameters and focus on candidate genes in order to define their level of expression during the various stages of acquiring the addictive behavior.

Massey, Steve- ***Bioinformatics and comparative genomics***

Dr. Massey's Bioinformatics Laboratory is involved in a variety of research projects including: (1) Comparative genomics of DNA repair genes in bacteria to test the influence of genome size on the complexity of DNA repair in bacteria, with implications for the evolution of mutation rates. (2) Virus evolution and mutation rates to elucidate the factors involved in determining mutation rates in DNA viruses, with a focus on the nucleocytoplasmic large DNA viruses (NCLDVS) (3) Protein and RNA structural prediction to investigate the basic faced alpha helix (BFAH) motif and its mRNA binding partner found in cellular lysyl tRNA synthetase, which is involved in HIV pathogenesis. (5) Adaptive evolution of the *Helicobacter pylori* genome in amerindian populations which involves the identification of novel pathogenic factors in *H.pylori* by an examination of the Ka/Ks ratio on a genome wide level.

Ortiz-Zuazaga, Humberto- ***Megaprobe Genomic Analysis***

Dr. Ortiz-Zuazaga's laboratory has a research program in bioinformatics, focused on high throughput genomics. Data is obtained from collaborations with UPR and stateside researchers. Free online repositories of high throughput data such as Genbank and the Gene Expression Omnibus provide suitable benchmark data for comparison studies. The research laboratory will build computer systems that can locate millions of probes on gigabase stretches of DNA, adapting traditional bioinformatics search tools such as BLAST and traditional string search algorithms. Comparative genomics or "metagenomics" of million probe systems is another research area where students can apply graph-theoretic approaches to construct comparable sets of probes across genomes.

Papa, Ricardo- ***Development and Evolution of Butterfly Wing Patterns***

Dr. Papa's work explores phenotypic plasticity as a source of variation for natural selection and adaptation to work upon by combining pure genetics, phylogenetics, developmental cell biology, and functional genomics. The focus of the lab is understanding the molecular mechanisms at the base of the evolution of mimetic wing patterns. Students in the lab will be encouraged to develop broad-based, innovative thinking in evolutionary biology, while exploring the intricacies of wing pattern variations and pattern diversity. They will be trained to use new equipment and taught variety of techniques in the area of genetics, developmental cell biology, and functional genomics. While working in the lab, students will also learn

and have hands-on opportunities to practice: a) animal rearing ranging from food preparation and animal handling to crossing experiments designed to explore specific segregation of characters, b) basic and modern molecular techniques.

Peña de Ortiz, Sandra- ***Genomics of Learning and Memory***

Dr. Peña's lab focuses on the study of the neurobiological and genetic bases of cognitive processes relating to learning, memory, and emotions. There are two specific lines of study: (1) The role of DNA recombination and repair processes involving DNA endonucleases, ligases, and polymerases in long-term memory formation. Dr. Peña lab has identified a group of candidate target genes regulated in learning and memory by genomic rearrangement processes. (2) The role of Nurr1, an immediate early transcription factor regulated by the cAMP response element binding protein (CREB) and CREB-binding protein (CBP) in learning and memory processes. Nurr1 is required for the normal development of dopaminergic neurons in the mesolimbic region of the brain. Current studies are examining the importance of Nurr1 in the hippocampus for the processing of emotions.

Rosa-Molinar, Eduardo- ***Connectomics of the Vertebrate Spinal Cord***

In Dr. Rosa-Molinar's lab, a major driving force in synapses research is the development of new technologies that will assist in answering two fundamental biological questions about mixed synapses: how do they form and how are they remodeled? Work in the laboratory prepares trainees to conduct contemporary circuitry neuroscience research that will advance understanding of the relationship of synapses, single neurons, microcircuits and motor behavior. By employing neuroanatomical and imaging technologies to reveal neural circuitry and to identify emergent properties, they have identified five different neural cell types in the spinal cord of the Western Mosquitofish, *Gambusia affinis* that work together in a distinct rapid copulatory neural circuit. However, the neurons within this sexually dimorphic rapid copulatory spinal neural circuit are intermingled, and even neighboring neurons of the same type differ in connectivity and function. REU-CRIB will work with a new chromagen reagent developed by Nanoprobes Incorporated to amplify a protocol for retrograde labeling of spinal neurons using a new neural tract tracer that crosses the gap junction portion of mixed synapses.

Rosenthal, Joshua- RNA editing in the nervous system

Research in Dr. Rosenthal laboratory focuses on RNA editing by adenosine deamination. The process is studied from different angles. First, how RNA editing affects the electrophysiological properties of ion channels and transporters. Second, structural modifications in RNA editing enzymes isolated from organisms that exhibit unusually high levels of editing. Recently, findings from these areas of investigation have led to new avenues. For example, the use of modified RNA editing enzymes that will target specific codons within specific mRNAs. In addition, the laboratory is interested in determining whether RNA editing is plastic, and if so, whether it can respond to environmental factors like temperature. Summer students will work on the identification of RNA editing sites by comparisons of DNA and RNA sequencing data.

Serrano, Adelfa-Role of the Glutathione Redox System during Plasmodium development.

Dr. Serrano's lab directs its efforts to elucidate the role of genes involved in glutathione (GSH) metabolism during malaria parasite development. The working hypothesis is that genes involved in GSH biosynthesis and homeostasis are key to parasite development and represent potential targets for interruption of the life cycle. The gamma-glutamylcysteine synthetase (ggcs) gene encodes the rate-limiting enzyme in the biosynthesis of GSH, while glutathione-S-synthetase (GST) and glutathione reductase (GR) keep the balance of oxidized and reduced GSH. One of the experimental approaches being used is to analyze mutants (pbggcs- and pbgr-deficient P. berghei parasite lines, and to develop and analyze other mutant parasites, such as GST- deficient) and compared them to wild type parasites at different stages by microarray expression profiling followed by proteomic analysis. Potential projects for the REU-CRIB student are the design and hands-on cloning and construction of plasmids for gene disruption in Plasmodium, participation in the genotypic and phenotypic analysis of mutant parasites, as well as on the analysis of expression of mutants showing arrest in development during the parasite's life cycle in the vertebrate and mosquito hosts.

Toranzos, Gary- ***Environmental Microbiology***

Dr. Toranzos' Environmental Microbiology Laboratory is involved in the characterization of a group of phages recently isolated using *Enterococcus faecalis*. This novel group of phages is being characterized morphologically as well as genetically. The possibility of horizontal transmission of genes including antibiotic resistance as well as virulence factors is being evaluated. These phages are being isolated from several different geographical locations and our lab is looking for the prevalence of the different types in the environment. The REU-CRIB student will take samples of marine and freshwaters and will analyze them for the presence of bacterial viruses and bacteria. Bacteria and viruses will be isolated and characterized using molecular techniques, as well as electronmicroscopy and traditional microbiology techniques. This project will also look at the possibility of using these viruses for phage therapy and characterize them as possible

microbial source tracking (MST) tools, since their ecology has so far shown that their only reservoir may be humans. These unique viruses will serve as a tool for indicating human contamination in recreational waters.

Vega, Irving E. – Neuroproteomics: proteome dynamics upon deletion of the EFHD2 gene

Studies in Dr. Vega's lab are aimed at identifying and characterizing proteome difference among specific brain regions and changes in response to environmental or cellular cues. Proteome dynamics could be defined as transient changes in post-translational modification, interactions, function and/or subcellular localization. However, if these epiproteomic changes are sustained, by cellular function, environmental conditions or insults, they could lead to cellular transformation. Taking this concept as a research approach the lab has identified specific post-translational modifications, protein-protein interactions and protein level changes associated to cellular cues at specific brain regions. A novel mouse protein named EFhd2 was identified as an associated protein of the microtubule-associated protein tau. EFhd2 is more abundant in the nervous system, although it is weakly detected in other tissues. This protein is conserved from nematodes (C. elegans) to humans. Thus, the laboratory is developing an EFhd2-knockout mouse to uncover its role in the development of the nervous system. At this point, heterozygous (-/+EFhd2) and homozygous (-/-EFhd2) knockout mice has been developed. The REU-summer students will work on high throughput technology (such as tandem mass spectrometry) and basic protein analysis techniques (such as protein-gel electrophoresis) to determine the effect that the deletion of EFhd2 gene has on the brain proteome. Moreover, the student will have the opportunity to learn how to dissect mouse brain tissue and identified specific brain regions and their respective function. At the end of the summer research experience the student is expected to present his/her research work and contribute to the understanding of proteome dynamics in the central nervous system.