BIOTECH CENTER
NEWS
UNIVERSITY OF ILLINOIS AT URBANA-CHAMPAIGN BIOTECHNOLOGY CENTER

Functional Genomic Analysis of Germ Cell Development in Planarians

Dr. Phillip A. Newmark is an Assistant Professor in the Department of Cell and Structural Biology. He received his Ph.D. in Molecular, Cellular, and Developmental Biology from the University of Colorado at Boulder and performed postdoctoral research at the University of Barcelona and the Carnegie Institution of Washington's Department of Embryology. He joined the UIUC faculty in August 2001.

Since the late nineteenth century, when Weismann proposed that an immortal germ line was propagated from generation to generation, producing a mortal soma at each generation, biologists have been intrigued by the question of how the germ cell lineage is established. In addition to serving as a link between generations, the germ cells represent an intriguing example of cellular differentiation, in which highly differentiated cell types maintain their totipotentiality and are capable of reproducing themselves indefinitely.

Two distinct modes of germ cell specification are generally observed in animals. In the most commonly studied invertebrates, *Drosophila* and *C. elegans*, maternally supplied cytoplasmic determinants localized to the posterior of the embryo specify the germ cell lineage early in embryogenesis. Likewise, localized determinants play a role in germ cell specification in many vertebrates (e.g., zebrafish, chickens, anuran amphibians). The specification of germ cells early in embryogenesis by localized maternal determinants has been termed "determinate" germ cell specification. Alternatively, many organisms utilize inductive interactions to specify their germ cells relatively late in embryogenesis. This "epigenetic" specification is observed in mammals as well as a wide range of basal invertebrate

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Professor Hans Bohnert is Interim Director of the W.M. Keck Center

When I arrived at UIUC more than 2 years ago, the existence of the Biotechnology Center, specifically the W. M. Keck Center for Comparative and Functional Genomics, provided a significant attractor. The instruction, general help, and specific trouble-shooting for my lab by the Keck folks have been superb. Who would not be impressed by the instrumentation and the expertise that is available? The Center supports the role of faculty as teachers of new technologies, provides high-throughput platforms, and it is my sincere hope that this will continue and expand even further. Recently, I have become involved in an advisory role with the Comparative and Functional Genomics unit. After a few months, I can say that the day-to-day performance is characterized by efficiency, competence and easy communication, managed by the directors in the individual groups (http://www.biotech.uiuc.edu/keck.shtml) and the Biotechnology Center administration (http://www.biotech.uiuc.edu/).

Taking stock, these URLs show the equipment and methodologies available, and—importantly—this equipment is efficiently used by people, who love to interact with whoever wishes to use these tools. In July 2003 a new DNA sequencer, the ABI3730, arrived that resulted in longer read lengths of sequences, a higher sequencing success rate, and faster turnover due to shorter run times. Also this summer, a second ABI3730, funded through an NIH Shared Instrument Grant, went on line. We have recently lowered sequencing costs as a result of the efficiency of these instruments. The Keck Center has highly competitive prices for DNA sequencing, analyzing the output, and database management. The

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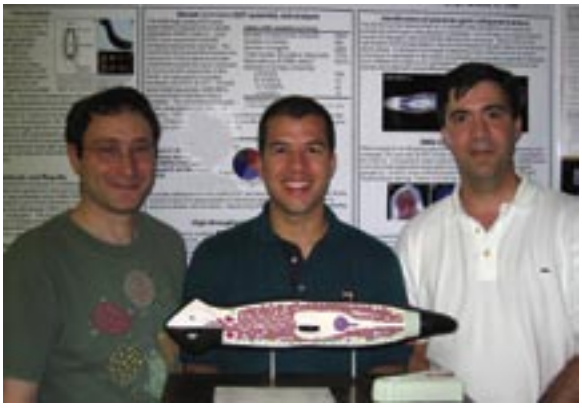
groups, such as sponges, cnidarians, and flatworms. Recently, tremendous strides have been made in understanding the mechanisms of germ cell determination; however, the molecular mechanisms that may link determinate and epigenetic specification of germ cells remain obscure. Ongoing work in the Newmark laboratory seeks to expand our understanding of epigenetic specification of germ cell fate using the freshwater planarian as a model organism.



Drs. Phillip Newmark, Ricardo Zayas, and Alvaro Hernandez (left to right)

Planarians are free-living, freshwater members of the phylum Platyhelminthes, the flatworms. Flatworms are among the simplest animals with three tissue layers (triploblasts) that display bilateral symmetry, an anterior concentration of neural tissue, and the organization of specialized tissues into organs. Planarians reproduce either asexually, by transverse fission, or sexually, as cross-fertilizing hermaphrodites. In the asexual mode of reproduction, the worm splits itself transversely into two fragments, each of which regenerates the missing tissue, thereby producing two planarians. In the sexual mode, hermaphrodites cross-fertilize, then lay egg capsules that contain several embryos. In sexually reproducing planarians the germ cell lineage does not appear to be segregated during embryogenesis; rather, when the planarian attains sufficient size, gonads and the copulatory apparatus are formed de novo in the appropriate regions of the worm.

Planarian (courtesy of Ricardo Zayas)

The planarians' remarkable regenerative abilities have led generations of biologists to study these organisms. When a planarian is cut transversely and separated into two fragments, the anterior fragment will regenerate a new posterior end while the posterior fragment will regenerate a new anterior end. The germ cell lineage shows a similar plasticity. Thomas Hunt Morgan showed that a planarian head fragment, completely devoid of any germ line structures, could regenerate functional gonads from the remaining somatic tissue. During the de-growth (shrinkage) that is the planarian's response to starvation, the gonads and copulatory apparatus are resorbed, only to be regenerated when the animal reaches the appropriate size after feeding is resumed. Following amputation of the head of sexually mature animals, the testes are resorbed and are only reformed after the regeneration of the head is complete. Thus, inductive influences are important for sexual differentiation in planarians.

The planarian *Schmidtea mediterranea* is the subject of our laboratory's studies. A tremendous advantage of using this species for studying germ cell specification and differentiation is that genetic differences appear to be responsible for determining the mode of reproduction. Thus, sexual and asexual strains of *S. mediterranea* can be distinguished by a chromosomal translocation present in the asexual strain. Individuals harboring this translocation reproduce only by transverse fission and do not differentiate germ cells, somatic gonad, or the copulatory apparatus; conversely, individuals lacking this translocation are hermaphroditic and do not reproduce asexually. The genome of this species is currently being sequenced by the Washington University Genome Sequencing Center in St. Louis.

With the goal of identifying genes required for germ cell specification and differentiation, we are working with the Keck Center for Comparative and Functional Genomics to generate a collection of expressed sequence tags (ESTs) from the sexual strain of *S. mediterranea* (supported by NSF CAREER Award IBN-0237825 to PAN). Alvaro Hernandez at the Keck Center has produced the normalized and subtracted cDNA libraries that are being sequenced. To date we have sequenced over 13,000 ESTs; bioinformatics analyses by post-doc Ricardo Zayas reveal that these ESTs represent over 6,500 unique transcripts. We will sequence ~20,000 ESTs that should represent approximately 9-10,000 unique transcripts. These ESTs will be invaluable for the annotation of the *S. mediterranea* genome.

The unique transcripts will be used to generate micro-arrays for examining global gene expression patterns in both sexual and asexual planarians, as well as during the process of sexual development. Genes that are upregulated in the sexual strain or during sexual development will be analyzed further by whole mount in situ hybridization. Automation of this technique permits high-throughput analysis of up to 96

different genes simultaneously, allowing us to visualize – at single cell resolution in the whole animal the cell types in which given genes are expressed. In this manner we will be able to identify genes expressed in the planarian germ cells. To examine the role that these genes play in germ cell development, we will use double-stranded RNA-mediated interference (RNAi) – a technique that is quite effective in planarians – to inhibit their expression and analyze the resulting effects upon germ cell development. Ultimately, these experiments will provide insight into the mechanisms by which germ cells are specified via inductive interactions and the extent to which these mechanisms have been conserved evolutionarily.

Dr. Phillip A. Newmark (pnewmark@uiuc.edu)

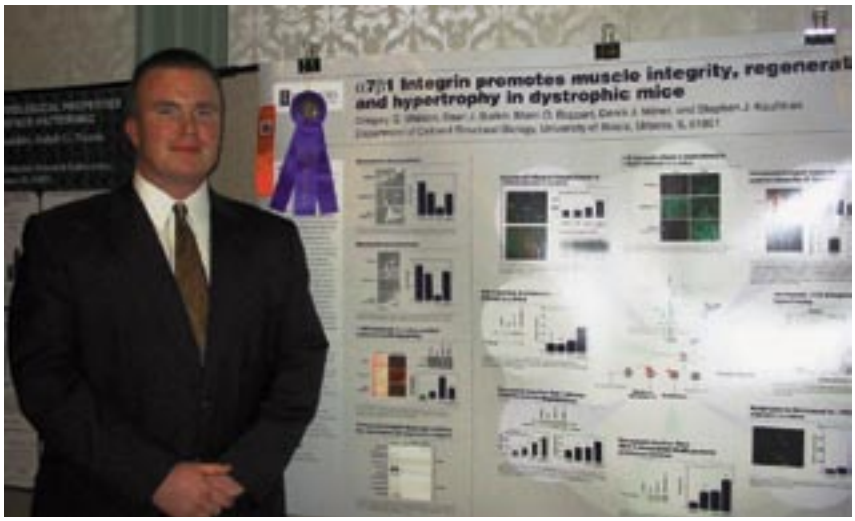
The June 2004 **Microarray Methods** workshop offered hands-on microarray experimentation and analysis. The BTC regularly offers several workshops including: Microarray Methods, Statistical Analysis of Microarray Data, Bioinformatics, Biacore Basics, and Flow Cytometry. These workshops focus on theory, experimental protocols, and data analysis to assist users in applications of the BTC's instrumentation to their own research projects.

2004 Catherine Conner Outstanding Dissertation in Biotechnology

The Biotechnology Center has established the *Catherine Conner Outstanding Dissertation in Biotechnology* award in honor of Catherine Conner's campus service. Catherine Conner retired as the Biotechnology Center's Placement Director in 2000 after 12 years of service. This annually recurring award is funded through a foundation established through the generous gifts of Eli Lilly, GlaxoSmithKline, Colgate-Palmolive, Monsanto, and Merck.

Each fall UIUC graduate students who are within one year of finishing their Ph.D. dissertations that focus in the areas of biotechnology, plant, microbial or animal genomics, functional genomics, proteomics, bioinformatics, or bioengineering are encouraged to participate. The posters are due in December and the winner is presented a $1,000 monetary award at the January Biotechnology Job Fair. We encourage qualified graduate students to call the Biotechnology Office at (217) 333-1695 for further information.

The 2004 Catherine Conner awardee is Greg Wallace. Greg is a Ph.D. student of Professor Stephen Kaufman in the Department of Cell and Structural Biology. Greg's award winning poster is entitled "α7β1 Integrin Promotes Muscle Integrity, Regeneration, and Hypertrophy in Rescued Dystrophic Mice."



Gregory Wallace the 2004 Conner Outstanding Dissertation in Biotechnology awardee.

Professor Hans Bohnert is Interim Director of the W.M. Keck Center, continued from page 1

facilities for generating and analyzing spotted oligo and cDNA microarray slides and the Affymetrix GeneChip workstation have been upgraded during the last year. Utilization by the community has picked up; soon there will be need for more instrumentation. New tools for data analysis, training opportunities and workshops make this unit the genomics hub for the campus community.

Also, a new service for creating custom normalized and subtracted cDNA libraries that greatly reduce sequencing redundancy (http://www.biotech.uiuc.edu/customlibrary.html). This service originated from the recognition that faculty, not restricted to Illinois, will benefit from expertly constructed, quality-controlled, and certified gene libraries. From my own experience such libraries reduce project cost—measured as the number full-length sequences and unigenes—by at least an order of magnitude. The service, with competence that comes from experience, will free individual labs from having to acquire the appropriate expertise. The Center's education and training mission includes that you construct your favorite cDNA (and other) library under the watchful eyes of the experts.

These examples document expertise and excellence of the Center for Comparative and Functional Genomics. However, a facility such as this is doomed if it were to rest on its laurels. We have to think about where we would like to be next year, and in five years. Science is driven by new technologies. These days new tools are conceived and implemented within a few years or even months. As a service facility the W.M. Keck Center has to play ball or become outclassed and unnecessary and this means upgrading and changing constantly.

Here is a list of suggestions for the Center to prepare for. Easy to predict is high-throughput DNA sequencing to accelerate, with new technologies that will streamline the pipeline from clone to sequence into more highly automated processes. The Center will have to adjust to such changes in sequencing to continue to serve the university. The computational pipeline from sequence to storage, analysis and integration into databases must be streamlined and results integrated into existing databases automatically. We must participate in the development of new high-throughput platforms, and new ways to visualize, measure and quantify gene structure, transcript expression, protein complexity, protein-substrate and metabolite interactions, and providing these tools to the community. Miniaturized and improved versions of platforms that are themselves only a few years old are coming on line.

Let me illustrate with the following example. Even though we have complete genome sequences for a few model organisms, it is recognized that populations, cultivars and ecotypes of species can differ in gene complexity and, even more so, in gene regulation. Large-scale genotyping, SAGE and SNP-analysis, anchored on a model system genome sequence, will have to fill in gaps that describe the diversity of related species or population variants of model genomes. Diversity will also require, for example, GeneChip experiments to be done in multi-well format, each well with a subset or all genes printed or synthesized, to advance comparative genomics. High-throughput quantification by real-time PCR will become a platform similar to microarray/GeneChip hybridizations. Conceivably, one service by the Center can be the design, synthesis, distribution, analysis and statistical examination of quantitative transcript expression in 384-well or even larger formats. Predictive computational tools for gene expression control, transcript maturation/splicing, posttranscriptional modification or interaction propensity will generate greater demands on the bioinformatics unit.

I imagine sequencing more model genomes to be in the near future, and the need for cultivar-, population-, and related-species-specific gene collections is obvious and so is the application of high-throughput tools for analyses of biodiversity. Generating and maintaining BAC/YAC/TAC-based gene collections, and their partial

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sequencing, for populations of organisms might well become a task for the Center.

Another area of rapid technological and conceptual change center on the integration of different platforms, which will require enhanced interactions between several units in the Biotechnology Center. An important part to satisfy this requirement will be expanding our bioinformatics capacity, developing strong interactions with the academic departments in computational science and engineering, and enhancing those interactions that exist. In fostering such interaction, as much as in the support of 'wet lab' experimentation, I also imagine increased demand for the Center's mission in training, consultation and advising on prudent experimental strategy. I hope it is obvious that I am glad to serve the unit and community.

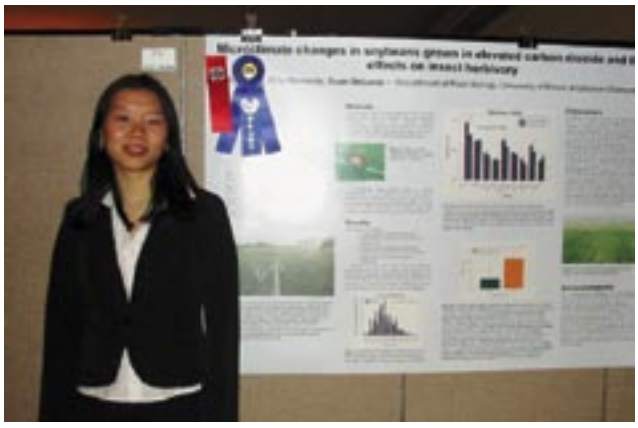
Dr. Hans Bohnert (bohnerth@life.uiuc.edu)

Affymetrix Workstation Upgrade

Affymetrix 3000 Workstation for processing and scanning GeneChips.

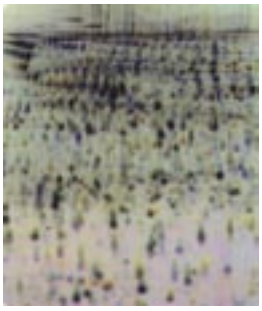
The Functional Genomics Unit recently updated its Affymetrix system to the new GC3000 GeneChip Scanner bringing improved performance, higher resolution and greater throughput to Affymetrix experimentation. The GC3000 scanner is compatible with higher density GeneChips used in new organism releases that was not compatible with our previous scanner. In addition, the Affymetrix GC3000 will enable use of human, mouse and rat genomes each on a single high density GeneChip. Further cost savings have been realized by Affymetrix recently pricing The University of Illinois for a GeneChip volume discount. Please contact Dr. Mark Band (markband@uiuc.edu, 217.244.3930) to discuss applications of GeneChip technology to your research. Assistance in design and analysis of Affymetrix experimentation is available from Dr. Lei Liu Director of Bioinformatics. Dr. Liu can be contacted at 217.265.5061 or email leiliu@uiuc.edu.

The Custom Oligonucleotide Synthesis Facility at the University of Illinois Biotechnology Center, offers a wide variety of oligo synthesis from scale sizes to specialized modifications, produced on the four column 394 ABI Synthesizer. Scale sizes offered are 40nmol, .2umol, 1umol, and 10umol. OPC and PAGE purifications are available at the 40nmol and .2umol scales. Our more common modifications are dye labeled oligos such as 6-FAM, TET, HET, Cy3 and Cy5. Standard and special ratio ambiguous bases are available, along with many other unique modifications. Call Lorie Rickords or Virginia Lukas at 217.244.1007 for further information.



Cathy Chaoran Yu was awarded $200 for the best undergraduate Student Science Poster. Ms Yu's Poster was entitled *Microclimate Changes in Soybeans Grown in Elevated Carbon Dioxide and Their Effects on Insect Herbivory*. The award was presented at the 2004 Biotech Job Fair last January at the University of Illinois. The Kimberly-Clark Company, Pierce Biotechnology, Inc., and the Biotechnology Center Placement Office sponsored the Poster Competition.

High Throughput Proteomics at Biotechnology Center



DIGE Gel labeled with CyDyes˙ Cy2, Cy3, and Cy5.

The instrumentation for high throughput proteomics at the Protein Sciences Facility was recently acquired using funding provided by a Roy J. Carver Charitable Trust sponsored scientific instrumentation grant. The newly acquired instrumentation includes an Ettan 2-D gel electrophoresis apparatus, a Typhoon™ 9400 multilaser scanner for gel imaging/analysis, a robotic sample handling workstation, and an Ettan MALDI ToF Pro mass spectrometer (MS). A number of high throughput proteomic research projects are currently under way and more are planned.

The 2-D gel electrophoresis is with an Ettan IPGphor Isoelectrofocusing first dimension separation followed by a DALT*six* electrophoresis second dimension separation. With the Ettan DIGE (Fluorescence Difference in Gel Electrophoresis) system samples are labeled with CyDyes™ (Cy2, Cy3 and Cy5) and multiplexed for co-separation during 2-D gel electrophoresis. The samples, usually a treatment and control, are each labeled with either Cy3 or Cy5; plus a Cy2 labeled internal standard for each treatment. The Cy2 labeled internal standard is an excellent way to remove gel-to-gel variation. Following 2-D electrophoresis, gels are scanned with a Molecular Dynamics Typhoon™ 9400 Imager with 3 direct excitable lasers and DeCyder™ Differential analysis software automatically detects, matches, and analyzes spots for very small differences in protein abundance between the two samples (Cy3/Cy5) on the same gel and between gels through Cy2 internal standards. After DeCyder™ analysis identifies gel spots for picking the gel is transferred to the Ettan Sample Handling Workstation (SHW). The SHW consists of a video camera and three robotic units working together to excise selected protein spots from gels for preparation for subsequent analysis and identification. The gel plugs are then destained, washed, trypsin digested, and spotted on a MALDI target. The SHW can be

Research Specialists Karen Avenatti and Jennifer Showerman using Ettan sample handling workstation.

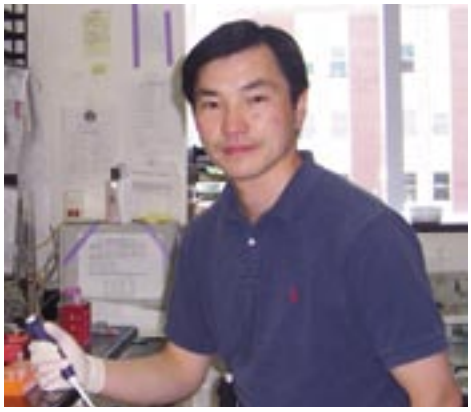
programmed to process up to 1152 samples per batch from a maximum of 12 large format 2-D gels in less than 36 hours. For mass spectrometry analysis we utilize an Ettan MALDI ToF Pro MS that can process 288 samples/batch and generate a protein identification list of the spots in about 6 hours.

Another approach is to use multiple dimensional protein identification technology (MudPIT) to separate and identify proteins. MudPIT is a liquid chromatography based method capable of resolving a mixture of digested peptides using tandem ion-exchange chromatography with reverse phase chromatography coupled with electrospray ionization MS (e.g. Thermo LCQ, Micromass Q-ToF). In collaboration with the Mass Spectrometry Laboratory at the School of Chemical Sciences, we are able to offer MudPIT analysis for complex protein samples. The Protein Sciences Facility also offers Edman Sequencing, standard 2-D gels, HPLC, FPLC, and peptide synthesis services. We are planning a short workshop in proteomics methodology that will cover these and other protocols and applications.

Dr. Peter Yau (pmyau@uiuc.edu) is Director of Proteomics at the Biotechnology Center

Ryan Kim, New Director of High-Throughput Sequencing and Genotyping Unit of the W.M. Keck Center for Comparative and Functional Genomics

In February Dr. Ryan Kim and his family arrived in Champaign from sunny La Jolla California in time to experience an Illinois winter. Ryan Kim earned his Ph.D. in Molecular and Cell Biology from the University of California at Davis in 1997. Dr. Kim was previously Senior Research Scientist and Manager of the Genomics Core at the Sidney Kimmel Cancer Center at La Jolla.



Dr. Ryan Kim

It is a great pleasure to be a part of the highly spirited, skilled, and dedicated DNA sequencing team of the High-Throughput Sequencing and Genotyping Unit here at the W.M. Keck Center. I have extensive experience in molecular biology and genomics in both plant and animal systems. During my post-doctoral training at Michigan State University my research included genome-wide expression profiling on regulatory genes that control the biosynthetic pathways of vitamin A and E in the marigold petal by using high-throughput sequencing of a petal EST library and global gene expression profiling using spotted DNA microarray. This project resulted in identification of developmentally regulated genes and assignment of new functions to previously unknown genes. Many of these newly discovered genes are tightly regulated during petal development and may provide insight into their role in secondary metabolism. Most recently, as a senior scientist and manager of Genomics Core at Sidney Kimmel Cancer Center in San Diego, California, I acquired expertise using high throughput applications of genomics in cancer research. I investigated and established high throughput genotyping procedures (in situ PCR and extension on microarray slide) to determine SNP genotypes of ≥8,000 human DNA for prognosis of ovarian cancer. I also worked on the development of high throughput analysis of promoter activity and RNA stability using microarray technology (Affymetrix GeneChip, cDNA-, oligo-, and promoter array) for the prognosis of prostate and ovarian cancer.

The W. M. Keck Center for Comparative and Functional Genomics offers comprehensive services in DNA sequencing and genotyping, functional genomics, and bioinformatics. The Sequencing and Genotyping Facility is fully staffed and equipped to perform high throughput and core DNA (plasmid, BAC, and PCR products) sequencing, genotyping, synthesize custom cDNA (normalized and subtracted plasmid) libraries, and custom oligonucleotide synthesis (including modified oligos). Recently we acquired second ABI 3730XL capillary sequencer. This sequencing platform offers state-of-the-art sequencing by yielding longer read lengths, higher success rates at lower input costs compared to previous sequencers. This acquisition has substantially expanded our sequencing capacity and contributed to lower pricing for our sequencing services.

We offer quick turn around (often next day) in both our core DNA sequencing (small scale sequencing and primer walking) and oligonucliotide synthesis services. We are continuously improving and customizing our protocols to expand high throughput capacity to be more cost effective and offer higher quality DNA sequencing. Please feel free to stop by and visit the laboratory. I look forward to working with you and wish you continued success and growth of your research program.

Dr. Ryan Kim (wrkim@uiuc.edu)

The High-Throughput Sequencing and Genotyping Team

Biotechnology Center Personnel

Evette Vlach

This summer both Allison Reese and Evette Vlach began working in the High Throughput DNA Sequencing Unit of the W.M. Keck Center. Ms. Evette Vlach earned her B.S. degree in Ecology, Ethology, and Evolution in 2000 from the University of Illinois. Evette was formerly at the Illinois Natural History Survey where she worked on West Nile Virus in the state of Illinois. In the Keck Center, Evette will be working on robotic colony picking using the Genetix Q PIX. Ms Allison Reese earned her B.S. in Animal Sciences, with a minor in Chemistry, in 2003 from the University of Illinois. Allison was formerly an Academic Assistant in the College of Agricultural, Consumer, and Environmental Sciences at UIUC. At the Keck Center, Allison will be working on both Core DNA sequencing and robotic plasmid preps.

Dr. Jyothi Thimmapuram

Allison Reese

Dr. Jyothi Thimmapuram has joined the Bioinformatics Unit of the W. M. Keck Center. Dr. Thimmapuram earned her Ph.D. in Physiology and Molecular Biology from the University of Illinois in 1996. She brings expertise in bioinformatics, functional genomics, and computer science to assist the users of the Keck Center.

Mr. Al Bari has accepted the position of Laboratory Supervisor in the Functional Genomics Unit. Al has extensive expertise in numerous robotic applications. He has a leadership role in maintaining and using the OmniGrid 100 in applications of printing oligonucleotide and cDNA spotted microarray slides.

Al Bari

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