BIOTECH CENTER NEWS

THE ROY J. CARVER BIOTECHNOLOGY CENTER OF THE UNIVERSITY OF ILLINOIS AT URBANA-CHAMPAIGN

Summer 2005

IN THIS ISSUE

Taking a Broader Perspective on Chondrocyte Gene Expression in Horses

Dr. James N. MacLeod recently accepted the John S. and Elizabeth A. Knight Chair at the University of Kentucky's Gluck Equine Research Center. Prior to his relocation to Kentucky in 2004, Dr. MacLeod was on the faculty for 11 years at Cornell University in the College of Veterinary Medicine. His undergraduate degree was earned in Animal Science at the University of Delaware, followed by veterinary school and a PhD in pathology at the University of Pennsylvania.

Synovial joints are composed of different tissues that function together to achieve movement with an amazingly low coefficient of friction between apposing bone surfaces. In health, joints move smoothly and pain-free with a full range of motion. Joint injuries, however, can cause inflammation and degenerative changes in multiple tissues including the joint capsule, synovial membrane, ligaments, articular cartilage, and the bone underneath the articular cartilage. Unfortunately, not all of these tissues have good regenerative properties. Cartilage, in particular, does not heal efficiently. The cells in articular cartilage (called chondrocytes) have only a limited capacity to repair structural defects in the joint surface. This is a primary reasons why osteoarthritis is a chronic and progressive disease. Lesions in the articular surface do not heal well and joint function often deteriorates further over time.

The prevalence of osteoarthritis is high in both humans and companion animals. One third of people report joint pain and stiffness sufficient to limit physical activity by the age of 45. The level increases to 75% for individuals that are over 65 years of age. With appropriate age adjustments, epidemiological data are similar in horses. The physical exertion and musculoskeletal stress of racing and other athletic events

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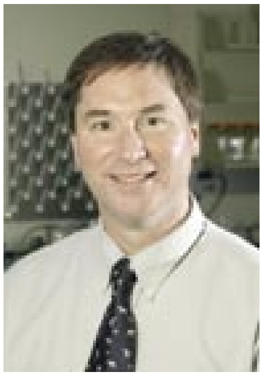
Roy J. Carver Biotechnology Center

The University of Illinois Board of Trustees approved the Chancellor's recommendation that the Biotechnology Center at Urbana-Champaign be renamed the Roy J. Carver Biotechnology Center, in recognition of the generous support the Roy J. Carver Charitable trust has provided for biotechnology facilities and research at UIUC. Since 1992, the Roy J. Carver Charitable Trust has provided nearly 12 million dollars of support to science and engineering initiatives at UIUC, approximately 8 million dollars of which has been directed toward biotechnology related research. Recently, the Carver Charitable Trust has funded 3 initiatives to the Carver Biotechnology Center: $500,000 for instrumentation to fund high throughput proteomics, $1,322,000 to create a Metabolomics Center, and $3,150,000 for development of bioinformatics and mass spectrometry.

Taking a Broader Perspective on Chondrocyte Gene Expression in Horses, continued from page 1



Dr. James N. MacLeod

that horses perform predisposes them to joint injuries. In fact, pain and lameness associated with joint disease is a primary variable that limits the athletic careers of racehorses. My laboratory conducts basic biomedical research on equine articular cartilage, primarily focused on the cell biology of chondrocytes. We study changes in chondrocyte gene expression that occur during normal development and with osteoarthritis, including a special interest in how cell/matrix interactions influence these events. There are many important questions to investigate. How does the normal function of chondrocytes change as a horse matures? How do healthy chondrocytes respond when a horse starts into heavy work and the biomechanical stresses placed on joints increase? What variables compromise the function of chondrocytes allowing structural lesions in articular cartilage to develop? How is chondrocyte function altered by medications and other therapeutic interventions? Why are chondrocytes normally unable to repair a lesion in the joint surface and fully restore the structural and biomechanical integrity of articular cartilage? If and when structural lesions in the joint surface develop, what can be done to enhance the regenerative potential of chondrocytes?

To investigate these and related questions, we have been working for the past three years with scientists at the Biotechnology Center to develop equine-specific genomic tools for the analysis of gene expression in horses. A normalized cDNA library was prepared from equine articular cartilage with Dr. Alvaro Hernandez. To date, 16,319 clones have been isolated and sequenced. Clustering analysis within the clone set defined 9,322 unique sequences (7,228 singlets and 2,094 clusters) and these were used to develop a cDNA microarray with Dr. Mark Band. Two projects are currently underway that include transcriptional profiling experiments. The first focuses on the maturation of articular cartilage, comparing chondrocyte gene expression between neonatal and mature horses (Figure 1).

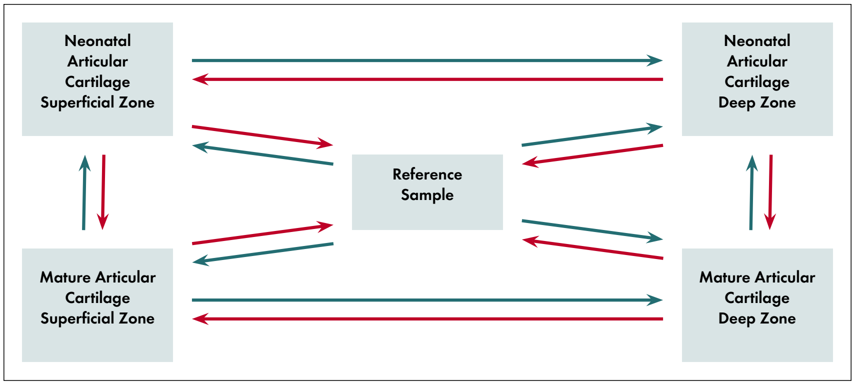


Figure 1

Real-Time PCR Instrumentation

ABI7900HT

The Functional Genomics Unit has recently upgraded gene expression capabilities with the acquisition of the Applied Biosystems 7900HT Real-Time PCR System. This upgrade enables higher throughput with low volume 384-well plates, and fully automated robotic plate loading, effectively extending hours of operation to 24 hours a day. This instrument has numerous applications that include gene expression quantitation and array validation or genotyping by detection of single nucleotide polymorphisms (SNPs). The Functional Genomics Laboratory offers training in use of this instrument, experimental design, primer design and trouble shooting. Additional information can be viewed at our website: http://www.biotech.uiuc.edu/centers/Keck/Functional_genomics/taqman.htm. Please contact Dr. Mark Band (217.244.3930, markband@uiuc.edu) or Tanya Akraiko (217 244 3929, akraiko@express.cites.uiuc.edu for assistance in using this instrument for your research.

In the second microarray project, we are studying fibronectin and how cell/matrix interactions regulate chondrocyte function. It turns out that cartilage contains a unique isoform of fibronectin that has an internal deletion of 771 nucleotides generated through alternative RNA splicing. To investigate the function of this cartilage-restricted variant of fibronectin, we are comparing gene expression in articular chondrocytes plated on different fibronectin isoforms. Finally, we are using the equine cDNA clone set for comparative mapping studies in order to help identify genetic determinants for traits of interest. Full genome sequence data are not yet available for the horse, but we are using putative equine/human orthologs and the program COMPASS to make chromosomal location predictions in collaboration with Dr. Lei Liu (Table 1).

Interactions with the Roy J. Carver Biotechnology Center continue to be a very helpful and important part of my research program. In addition to the individuals mentioned above, I would like to acknowledge and thank Peter Schweitzer, Al Bari, George Gong, Laura Guest, Robin Everts, Annelie Everts-van der Wind, Harris Lewin, Ryan Kim, and Mark Mikel.

—*James N. MacLeod (jnmacleod@uky.edu)*

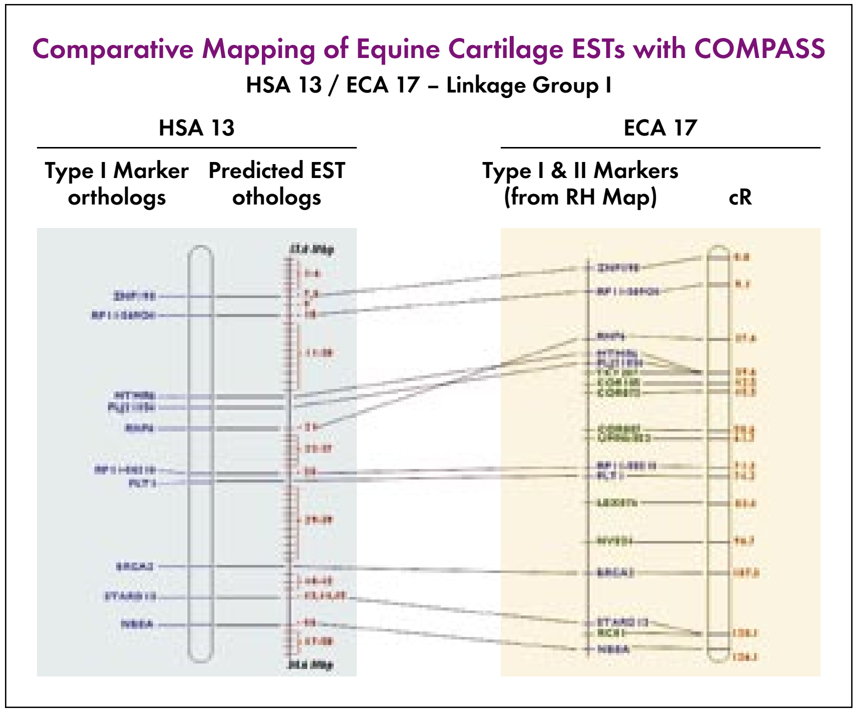


Table 1

NCBI Resources Workshop

Professor Katie Clark of the Biotechnology Library and the Carver Biotechnology Center has made arrangements to host the National Center for Biotechnology Information's (NCBI) course *A Field Guide to GenBank and NCBI Molecular Biology Resources* on September 22 and 23, 2005. This is a combination lecture and hands-on computer workshop on GenBank and related databases including use of Entrez databases and searching, BLAST search engine, genome data, and other resources.

The course will cover the following topics: GenBank database, NCBI derivative databases, searching using Entrez, and NCBI structure databases. As well as genomic resources at NCBI including: RefSeq, UniGene, variation data (SNPs), map viewer, human genome, mouse genome, rat genome, and microbial genomes. The course will feature the NCBI assembly and annotation of the human and mouse genomes, updated map viewer genome displays, the new genome specific BLAST WebPages, new NCBI curated conserved domains, and Cn3D4.1.

Further details can be found at http://www.ncbi.nlm.nih.gov/Class/FieldGuide/. The course is offered at no charge on campus at UIUC, but registration is required. The course has two parts: a 3 hour morning lecture followed by a 2 hour instructor led hands-on computer workshops in the afternoon. Please contact Ms.Rhonda Lipking (lipking@uiuc.edu, 217.333.1695) for details and enrollment.

Immunological Resource Center

Since the first applications of antibodies in scientific research a few decades ago, they remain a powerful tool for biological research. As we enter the post-genomic era there is increased interest in determining the biological function of genes. Using antibodies, scientists can readily study protein localization, structure, and function by performing various biological assays such as ELISA, western blot, immunohistochemistry, and immunoprecipitation.

An Antibody is a large soluble serum protein called an immunoglobulin. It is produced by the B lymphocyte of the animal immune system as a defense response to a foreign molecule or antigen. Theoretically any molecule that is non-native to the host can trigger the host's immune system to make an antibody specific to that antigen. Antibodies bind to a specific antigen to form an antibody-antigen complex, which can lead to antigen disintegration by other immune cells *in vivo*. The antibody-antigen complex can be directly detected by analytical means or indirectly detected by color change or fluorescent radiation through a secondary antibody conjugate. Therefore, antibodies provide researchers a tool to selectively detect a target molecule, whether it is a protein, DNA, lipid, or other types of molecules. Although genetic engineering using phage or yeast display technology has allowed scientists to manipulate antibodies, the best methodology for antibody synthesis is using an animal's immune response *in vivo.* Antibody production in animals is by far the most efficient and reliable method for creating highly diversified and specific antibodies. The Immunological Resource Center (IRC) assists campus researchers in making both polyclonal and monoclonal antibodies.

What is a polyclonal antibody? Polyclonal antibodies are a pool of antibody molecules with different specificities to a particular antigen. They are found in an immunized animal's blood and can be harvested in a serum form. Thus anti-serum often refers to crude polyclonal antibody. A polyclonal antibody usually has a high avidity due to signal amplifications by different antibody species bound to various parts of an antigen. Besides exhibiting strong signals, polyclonal antibodies are quicker and less expensive to produce than monoclonal antibodies, thus are more commonly used. The IRC currently offers polyclonal antibodies produced in rabbit, mouse, rat, and chicken. Chicken antibody is usually purified from egg yolk where antibody concentration is comparable to that of serum antibodies.

What is a monoclonal antibody? In contrast to a polyclonal antibody, a monoclonal antibody contains clones of a single antibody species of the same specificity to one epitope of an antigen. This single specificity of a monoclonal antibody makes it unique for doing many things that can't be achieved with promiscuous polyclonal antibodies. Monoclonal antibodies can give an unambiguous clean signal and this is useful for mapping epitopes, identifying isoforms, performing functional comparison of closely related proteins, or conducting protein structure studies.

How does one create a monoclonal antibody? Monoclonal antibodies are made by hybridoma technology invented by European scientists Milstein and Kohler in 1974, for which they shared a Nobel Prize in Medicine in 1984. To make a hybridoma, basically, an immunized mouse or rat is sacrificed and lymphocytes are isolated from spleen and fused with a mouse tumor cell line using the chemical polyethylene glycol. The resulting cells from this fusion are grown in a selective medium that will kill non-fused cells and leave only hybrids of lymphocytes and tumor cells survive. Multiple-cell fusions often lead to the death of that hybridoma. After several rounds of screening the culture medium against antigen in biological assays, a stable immortal hybridoma producing only one monoclonal antibody will be selected. In one fusion, several monoclonal antibodies of different specificities can be produced. Since a hybridoma cell line will constantly secrete a monoclonal antibody to its medium, hybridoma conditioned medium is thus a form of crude monoclonal antibody. The hybridoma can also grow in the mouse peritoneal cavity to make ascites fluid that contains high concentrated monoclonal antibody. A hybridoma cell line can be frozen for long-term storage and thawed out when more of the monoclonal antibody is needed, thus providing a great convenience to researchers. IRC has vast experience making custom monoclonal antibodies in mouse and rat at competitive prices.

Many researchers today prefer to use a peptide antigen to make antibodies. This method has a great advantage when a gene sequence is known, but a gene product is not available or a target protein shares a large homology with other protein(s) in the same antibody assay system. Peptide antibody can give a signal that is as strong as a protein antibody and peptide antibodies can be made as either a polyclonal antibody or a monoclonal antibody. However, there are a few potential risks of using a peptide antigen. One is that a peptide sequence can overlap with other protein sequences therefore an antibody product of a peptide antigen may be cross-reactive with other proteins; secondly, a linear peptide sequence might not produce antibody that recognizes a discontinuous epitope in target protein; and lastly, an antigen peptide selected based on a pure DNA sequence may not generate an antibody that recognize target protein in its native form because of unknown post-translational modifications. These pitfalls can be largely reduced if one knows how to design good peptide antigens, which need to combine the knowledge of protein structure, immunology, bioinformatics, and antibody production. The IRC has experience designing peptide antigens and subsequently we have successfully produced many excellent polyclonal and monoclonal antibodies. Our peptide design service is included in our antibody production package.

The IRC is committed to quality products, timely delivery, and user satisfaction. As a university core facility, our goal is to assist researchers in applications of immunology. We provide extensive consultation before a project is started and tailor each project according to the nature of an antigen and the ultimate antibody assay. We understand that a successful antibody project involves a good collaboration between the IRC staff and the researcher. For this reason, we offer complimentary training to our users in applications such as western blot, ELISA, immunoprecipitation.

Liping Wang with the Biacore 3000.

Biacore 3000 Service. The IRC hosts a Biacore 3000 instrument and provides instrument training and experimental assistance for researchers who wish to use this instrument. The instrument design is based on optical phenomenon surface plasmon resonance (SPR) to detect molecular changes on a sensor chip surface in real time. The methodology is highly sensitive and uses a small quantity of reagents; no molecular labeling is required for the analyte, and a sensor chip surface has four reaction flow cells for easy experimental setup. The biological sensor chip surface can be repeatedly used once it is made. To perform a Biacore experiment, one interactant (ligand) is immobilized to a sensor chip surface through a chemical reaction or through a non-covalent capturing method. The second interactant (analyte) is then delivered to the sensor chip surface through a liquid transporting system to interact with the ligand. Signals derived from molecular bindings will be converted into response units, which are plotted against reaction time. Major Biacore applications include affinity measurement, reaction rate determination and concentration assay. The Biacore 3000 can also perform analyte recovery from the sensor chip for MALDI analysis providing a unique tool for the identification of unknown binding proteins to a known ligand.

The IRC's antibody and Biacore services are available in 264 Burrill Hall, 407 South Goodwin Avenue, Urbana, IL 61801. Liping Wang, Laboratory Supervisor, can be reached at (217) 244-0557 or lpwang@life.uiuc.edu for further discussion on applying immunology to your research. Pricing and major service information is available at our website at http://www.biotech.uiuc.edu/centers/IRC/

—Liping Wang

The Colgate-Palmolive Company awarded five outstanding UIUC graduate students, each receiving a fellowship award of $3,000. These students are in their last year of graduate studies from various departments including Biochemistry, Chemistry, Food Science and Molecular Biology and have an interest in a career in the consumer products industry. We appreciate Colgate's support of our exceptional students at the graduate level. Congratulations to the winners. This award is facilitated by the Roy J. Carver Biotechnology Center's Placement office.

The Colgate-Palmolive winners are (left to right): Jeff Turner, Amir Mirarefi, Farah Jean-Jacques Toublan, Julie Akana, Rebecca Coppins.

Catherine Conner (right) presenting award to Matthew Gordon.

2005 Catherine Conner Outstanding Dissertation in Biotechnology winner is Matthew Gordon whose winning poster is entitled *Single Molecule Fluorescence Haplotyping by DNA Barcoding.* Catherine Conner presented a $1,000 monetary award and certificate to Matthew at the UIUC Biotechnology Job and Information Fair. The Biotechnology Center has established the *Catherine Conner Outstanding Dissertation in Biotechnology* award in honor of Catherine's campus service. Catherine retired as the Biotechnology Center's Placement Director in 2000 after 12 years of service. Matthew Gordon is a graduate student of Professor Paul Selvin.

We wish to thank Drs. Mark Band, Hans Bohnert, Jennifer Eardley, Robert Gennis, Louis Hoyer, Neil Kelleher, and Peter Yau for dedicating time to judge the 18 submitted posters. This award is made possible through the generous contributions of Colgate-Palmolive Company, Eli Lilly and Company, GlaxoSmithKline, Merck and Company Inc., and Monsanto Company.

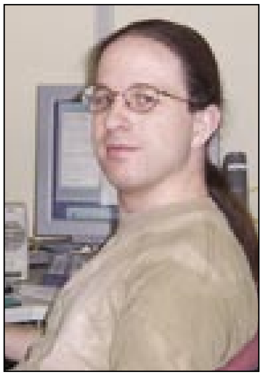
All UIUC Ph.D. candidates whose studies are within the area of molecular biology, genomics, proteomics, or other applications of biotechnology and within a year of completion of their dissertation are invited to participate. Qualified candidates are required to submit a poster presentation (sized at or between 3'x 3' to 3' x 4') and one page typed abstract.

Integrated Bioinformatics and Proteomics

The Roy J. Carver Biotechnology Center (CBC) recently received a $3,149,000 funding for bioinformatics and mass spectrometry from the Roy J. Carver Charitable Trust. The proposal of Professors Jonathan Sweedler and Neil Kelleher will provide funding for a superconducting magnet to establish a 15 Tesla Fourier-Transform Ion Cyclotron Resonance Mass Spectrometer to enable UIUC to have state-of-the-art instrumentation for proteomic and metabolomic experimentation. The acquisition of the FTMS capabilities will allow greater proteome coverage with its high mass capabilities, increased throughput, more fragment ions, highly accurate mass values, and applications of *accurate mass tag* approach.

Bioinformatics funding will fund three bioinformatics specialists in gene expression, proteomics, and metabolomics experimental design and analysis. These specialists will work closely with users of our Functional Genomics, Protein Sciences Facility, and Metabolomics Center and work as a team in unifying data across all three centers where they apply to a given project. Provided funding will also enable us to upgrade our bioinformatics software and computer infrastructure. This funding facilitates the creation of an integrated bioinformatics resource and world class proteomics measurement capabilities to the UIUC research community.

New Roy J. Carver Biotechnology Center Personnel

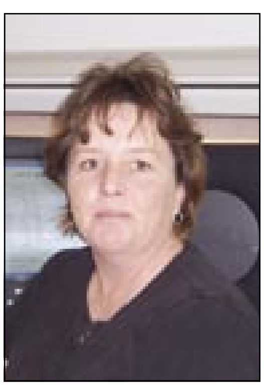


Lee Bynum

Lee Bynum has started in the Bioinformatics Group as a research programmer. Lee is a recent graduate of UIUC with a B.S. in Software Engineering. Lee will be maintaining and modifying our proprietary software systems: ESTIMA, Pipeline, and GPMS. Lee has accumulated significant software writing experience working as an undergraduate for Professor Neil Kelleher on ProSight PTM which is a protein identification software.

Tatsiana Akraiko

Tatsiana Akraiko has joined the Functional Genomics Group as a Research Specialist. Ms. Akraiko recently finished her M.S. degree at UIUC in Crop Sciences working for Professor Kris Lambert. Tatsiana has extensive experience in real-time PCR experimentation and will be working with our ABI7900HT Taqman instrumentation. In addition, she will be assisting users with Affymetrix GeneChip experimentation, robotics in liquid handling and microarray printing.



Nina Stadler

Nina Stadler is working at the W.M. Keck Center as a Secretary III. Nina is involved in general secretarial duties as well as assisting the staff with purchasing.

Dr. Jenny Drnevich

Dr. Jenny Drnevich was recently hired as a Functional Genomics Bioinformatics Specialist. Dr. Drnevich has earned her B.S. from Purdue University and a Ph.D. from Arizona State University. Jenny has been a postdoctoral research associate working with Professor Kimberly Hughes at UIUC. Dr. Drnevich will be assisting researchers in oligonucleotide microarray, cDNA microarray, and Affymetrix GeneChip experimental design and statistical analysis.

Dr. Kelly Keating

Dr. Kelly Keating is working in the Metabolomics Center as a Senior Research Specialist. Dr. Keating has earned her B.S. in Chemistry from the University of Wisconsin and her Ph.D. in Chemistry from the University of California, Davis. For the past 6 years Dr. Keating has been a Senior Research Scientist with Wyeth Research in Collegeville PA performing small molecule and metabolite structure elucidation and was manager of their Spectrometry Services. Kelly will be assisting the users of the Metabolomics Center with there research projects.

Roy J. Carver Biotechnology Center at the University of Illinois at Urbana-Champaign

Jonathan Sweedler, PhD • Director and Lycan Professor of Chemistry

Mark Mikel, PhD • Associate Director and Editor

Hans Bohnert, PhD • Director of the W.M. Keck Center for Comparative and Functional Genomics and Professor of Crop Sciences and Plant Biology

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Metabolomics Center

Dr. Mengfei Ho, Interim Director of the Metabolomics Center

The Roy J. Carver Biotechnology Center has opened a Metabolomics Center to assist users in isolation, identification, and quantification of metabolites. The Metabolomics Center is located in C301 Chemical and Life Sciences Laboratory at 601 South Goodwin Avenue, Urbana. The primary goal of the Roy J. Carver Metabolomics Center is to measure and identify metabolites and small molecules by using multiple complementary analytical methods. The Center is equipped with GC-MS, HPLC-MS, HPLC (stand alone), Piezorray robotic printer (non-contact microarray printing onto membranes, plates, and slides), ultraviolet/visible/fluorescence microplate reader, microplate chemiluminometer, robotics for colony picking and re-array, and a chemosta/bioreactor fermentation system. The instrumentation for this Center was funded through the generosity of the Roy J. Carver Charitable Trust in funding an instrumentation proposal of Professor Brenda Wilson (Microbiology) and her colleagues.

Users (both UIUC on-campus or off-campus) can use these services and instrumentation in their experimentation after sufficient training. Depending on instrumentation and user preference there are several categories of user/staff participation: User walk-up, user operation (after training by staff), full service by the Center's Staff, and collaborative projects where the investigator and the Metabolomics Center staff partner in experimental design, experimentation, troubleshooting, and data analysis. Planning is under way to add software for Metabolomics data analysis as well as adding a bioinformatics specialist.

Please contact Dr. Mengfei Ho, Interim Director, at (217) 333-5939 or mengho@life.uiuc.edu for assistance in using the center for your project.

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