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The University of Oklahoma HEALTH SCIENCES CENTER

In this issue

Acknowledgements

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Technology seminars to illustrate new equipment

This fall, we are sponsoring three technology seminars that will be offered during the regular seminar series for the Department of Microbiology and Immunology. These seminars, at noon on Mondays, are designed to bring you up to date on new technology that we offer in our core facility or technology that we are thinking about adding to our repertoire. On September 25, Dr. Wendy VanScyoc will discuss the use of surface plasmon resonance ("Next Generation Surface Plasmon Resonance Biosensor Technology for the Production of High Quality Kinetic Information in Protein Interactions"). On October 9, Dr. Ed Horton will discuss how you can take advantage of our Beckman Proteomelab ("Applying Intact Protein Partitioning and Fractionation to Biomarker Discovery"; see related article on page 3). Lastly, Dr. Charles Greaves will discuss on October 30 a new microarray technology that we are considering ("Custom Microarrays for Application Specific Studies"). We hope that you will be able to attend, as we will be sending around surveys after each seminar, assessing how useful this has been, and whether you are interested in having these technologies in the core facility. Thanks for your support!

-Dave Dyer & Allison Gillaspy

Featured project: InterGenetics SNP Genotyping

InterGenetics Incorporated (IGI) is offering a new service to provide high throughput single nucleotide polymorphism (SNP) genotyping to academic investigators and biotechnology companies. IGI is a biotechnology- and bioinformatics-based company in the PHF Research Park located adjacent to the OUHSC. IGI has significant expertise in developing and implementing multiplexed SNP assays through its development of a test for genetic predisposition to breast cancer. Over a million individual SNP genotype determinations were made by IGI over the course of this and other projects. IGI is making available this expertise in SNP genotyping to other investigators.

Initiating and operating a large-scale SNP genotyping project can be expensive and time consuming. Capital equipment costs are large and technical operators usually require extensive training. IGI allows you to avoid these costs, shortening the time required for data acquisition and permitting you to concentrate on data analysis and interpretation. IGI's core technology uses allele specific primer extension (ASPE) mounted onto a bead-based assay system with a readout linked to a flow cytometer. This robust technology uses the same operating platform as that for the only FDA approved cystic fibrosis mutation carrier screening test.

One of the factors that makes this new service possible is access to robust, accurate and timely DNA sequencing provided by the OUHSC core facility. Development of accurate SNP genotyping assays using ASPE requires a modest sample of individuals for whom SNP genotypes have been determined by a method other than ASPE. It also is critical to know the locations of nearby SNPs, to avoid problems in PCR and/or ASPE primer design. Direct DNA sequencing is the gold standard for finding and characterizing such SNPs. Thus, development of each new assay begins with a small resequencing project.

If you are contemplating a SNP genotyping project, IGI will deliver timely, efficient and competitively priced user-friendly service. And, IGI technical staff will be glad to guide you through the process of designing your SNP genotyping application. Interested investigators can contact IGI at 405-271-1720 or visit their website at www.intergenetics.com.

Contributed by David Ralph, Ph.D.
CSO, IGI

INBRE News *by Edgar Scott, M.S.*

This summer, I had the pleasure of mentoring Craig Covey from Oklahoma City Community College during the INBRE Summer Undergraduate Research Program (see page 5). His research focused on using bioinformatic tools to identify unique sequence motifs in a family of bacterial metal ion sensor proteins. The Ferric uptake regulator (Fur) protein and the Zinc uptake regulator (Zur) protein are related proteins that regulate the uptake of iron and zinc, respectively. Their amino acid sequences are so similar that it is difficult to distinguish between the proteins. Craig's project attempted to identify unique amino acid motifs from a group of Fur/Zur homologues that might be useful for distinguishing Fur from Zur.

Craig proceeded in several steps. He first acquired amino acid sequences of experimentally-characterized Fur and Zur proteins from the UniProt Knowledgebase. To this, he added sequences homologous to Zur and Fur using Protein Specific Iterative BLAST (PSI-BLAST) at NCBI. Through several iterations, PSI-BLAST creates and refines a specific scoring matrix that models the query sequence and sequence relatives and can be used in successive searches to find distantly related sequences. He then processed the two data sets separately with a motif searching program called MEME (Maximum Expectation maximization for Motif Elicitation). MEME uses the expectation maximization algorithm to identify conserved characters in a group of protein or DNA sequences. The program outputs conserved regions as local multiple sequence alignments with a bit score to quantify the degree of conservation within that alignment.

This analysis showed that the Fur and Zur proteins have a strong sequence motif that overlaps both the DNA binding domain and portions of a metal-binding domain. Unfortunately, the sequence logos of the motifs for the two proteins did not distinguish Fur from Zur. However, the exercise was good for Craig's introduction to bioinformatics and suggested several possible avenues for additional investigation.

Oklahoma INBRE
IDeA Network of Biomedical Research Excellence

http://okinbre.org/

COBRE Investigator Focus: John West, Ph.D.

Defining the impact of HIV-1 fitness on virus transmission from mother to child

Infections with HIV-1 subtype C are responsible for more than 50% of new infections and this continues to increase, particularly in the developing countries of sub-Saharan Africa, India and China. The primary routes of infection are unprotected heterosexual contact and mother-to-child transmission.

Untreated mothers transmit HIV-1 to their infants in approximately 30% of deliveries. Of the remaining 70%, 15% become infected through breast-feeding. My laboratory focuses on mother-to-child transmission because this represents one of few instances where the donor, recipient, the direction and timing of transmission are known.

Transmission of HIV-1 is a function of the envelope glycoprotein (Env) that mediates fusion of the viral membrane with a target cell. Because the viral replicase lacks proof-reading capability, the Env proteins are tremendously diverse, creating a swarm of nearly identical but distinct individuals called a 'quasispecies'. This diversity lends incredible plasticity to the virus to adapt to selective pressures. Recent data suggest a link between HIV Env and viral ability to survive in a given environment (fitness), and between fitness and disease progression.

We use a combination of genetic, phylogenetic, molecular, immunological and biochemical approaches to investigate relationships between Env evolution and biological function, including replication or transmission fitness. We employ fluorescent-tagged HIVs (containing variants of Env) in dual-infection competition experiments to define replication fitness. We also are developing organotypic culture systems to evaluate viral transmission fitness across mucosal barriers. These experiments will facilitate our understanding of the determinants of HIV-1 transmission, evolution and disease. Such information will be essential in the design of treatments or preventatives that limit or redirect viral Env evolution such that lasting protection can be achieved.

John West, Ph.D.
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The Beckman PF2D ProteomeLab in Practice

In our last newsletter, we announced the availability of the Beckman PF2D ProteomeLab for proteomics separations by our core facility. Bob Hurst's lab has extensive experience with the PF2D, improving the throughput and accuracy of the instrument. Bob and coworkers have a recent paper published online with Biomed Central's "Proteome Science": http://www.proteomesci.com/content/pdf/1477-5956-4-13.pdf. This article is a description of their experience, and advice on how to employ the PF2D for your own purposes. Bob writes:

"The power of proteomics is that it represents the working level of the cell. Important regulatory events involving post-translational modification may not be reflected at all in the levels of transcripts. The PF2D is certainly one of the most advanced proteomic displays around. The first dimension separation is by chromatofocusing, which separates by pI. The actual separation can be divided into three groups of proteins. First are the highly basic proteins that either are eluted immediately the by pH 8.5 wash or elute slowly in the next several fractions. The PF2D is set to wash out everything that doesn't bind strongly at pH 8.5, but some 10 or more fractions are collected. While many of these contain protein, most proteins are found in the first two fractions. Whatever separation occurs at this pH is poorly understood, and consecutive fractions will be seen to contain the same bands. The next set is collected during the pH gradient elution. This is where resolution is best; most proteins eluted in the gradient appear as well-resolved peaks in the second dimension. The third group of proteins is eluted at pH 4.0 by a 1 M NaCl wash. These highly acidic proteins are not well resolved, but the first two fractions generally contain most of the information.

To speed the proteomic display, we routinely run only 25 of the 44 first-dimension fractions through the second dimension. Also, to speed the separation, we do not collect fractions but just discard the material. Generally, sufficient material is collected from each first-dimension fraction to make two second dimension runs. Because a total of about 4,000 fractions would need to be collected if the entire first dimension were fractionated, and because the fraction collector is not large enough for that many fractions, someone has to change out the full tubes for empty ones. This either means someone does this at 4AM, or the fractionation requires some three days. Running in "mapping mode," we can complete a separation in 24 hours.

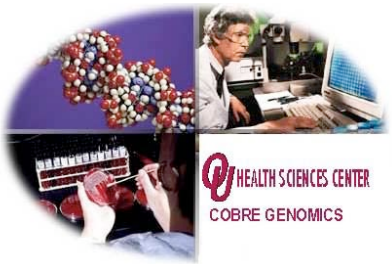
We then take our map and compare it to our control or controls to decide which peaks need to be identified by mass spectroscopy (MS). The identification of proteins by peptide mass fingerprinting is relatively simple, and there is no in-gel recovery needed. We were most interested in peaks that were unique to one sample in our study of the effect of extracellular matrix on the phenotype of bladder cancer cells. We picked the fractions we wanted to be analyzed by MS and then re-ran the appropriate first-dimension fractions, collecting the second-dimension fractions. We showed that even a small peak with an absorbance of 0.02 could easily be identified. We found that many of these unique peaks represented post-translational modifications and that large proteins are easily separated by this technique. Two of our proteins were >300 KD in size. The main source of error is pH control. If two first-dimension fractions differ slightly in pH coverage, then the second-dimension separations will be different. For example, if sample A yields a first-dimension fraction covering the range of 7.0 to 7.3, whereas the equivalent fraction from sample B covers 7.03 to 7.33, then the proteins eluting between 7.00 and 7.03 will be missing from the second-dimension separation of sample B and will appear to be unique in sample A. However, those proteins will appear in the adjacent fraction in sample B. It is therefore important to examine closely the adjacent fractions and the pH of the first-dimension fractions before deciding a protein is unique.

In summary, we first map a proteome and then pick fractions containing interesting proteins for subsequent examination. Clearly, it would be most exciting if the PF2D could be directly interfaced with the mass spectrometer. If anyone has questions as how to best use the PF2D, I will be glad to share my experience."

Contributed by Robert Hurst, Ph.D.

Beckman PF2D ProteomeLab proteomics separations platform

COBRE News *by John Iandolo, Ph.D.*



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In late July (21-23), NCRR hosted the Institutional Development Award (IDeA) Program First Biennial Symposium. The highlights of the meeting included presentations by NIH Director Dr. Elias A. Zerhouni, who spoke on "NIH at the Crossroads: Strategies for the Future"; a presentation by NCRR Director Dr. Barbara M. Alving, who provided an overview of NCRR and lastly a presentation by Dr. Fred Taylor, who provided an historical account of the IDeA program. Dr. Zerhouni's message focused on the present budgetary problems at NIH and the plan to rescue the NIH mission. The plan calls for utilizing adaptive strategies to preserve NIH key principles. These will include developing a balanced research portfolio with NIH continuing to focus on basic research and the private sector taking the lead in clinical and translational research, by protecting the future with imaginative new programs for new and established investigators and by better management of available grant funds to maintain a balance in supply and demand. Following these talks, the meeting provided extensive opportunities for researchers supported by INBRE and COBRE funds to present and discuss the results of their work in comprehensive poster sections and at specifically focused scientific sessions. Our COBRE was well represented with posters presented by Dr. Ira Blader, Dr. Michael Sakalian, Dr. W. Michael McShan, Dr. Holly Hoffman-Roberts, Dr. Mark Lang and Dr. John West. Following the scientific sessions, the annual COBRE Principal Investigator's meeting was convened. Announcement of a permanent study section to review proposals gave rise to a lively discussion of the review process for COBRE competitive renewals. In the open discussion session, the future of the COBRE program ranged over a number of topics. Among the most interesting, follow-on programs to continue support of successful COBREs generated much interest.

INBRE Summer Undergraduate Research Program

http://okinbre.org/

The INBRE Summer Research Program for Undergraduate Students recently concluded its fifth year of student participation. This year's program was the biggest yet, with thirty-one students participating from six regional universities and three community colleges. The goal of the program was to expose undergraduate students to the world of biomedical research and encourage them to pursue careers in the fields of science and technology. The students were matched with mentors who share their same area of interest.

At the end of the eight-week program, the students presented a poster of their research findings and attended a luncheon in their honor at the OU Health Sciences Center. Two award winners from the INBRE program were announced at this year's luncheon. Michael Landoll from Cameron University was selected to represent OU Health Sciences Center at Research Day at the capitol this coming spring. Research Day at the Capitol is an opportunity for undergraduate students to inform the Legislature and the public about high quality research being conducted at Oklahoma's colleges and universities. Also at the luncheon, INBRE student Kelly Etherton from Oklahoma City Community College received a $2000 transfer scholarship to attend OU this fall. The transfer scholarship is given to the community college student with the best poster and presentation skills.

Applications for the 2007 program will be available this fall for both students and mentors interested in the program. To learn more about the INBRE Summer Research Program or the Oklahoma INBRE grant, please visit the INBRE website at www.OKINBRE.org. Students who participated in this summer internship, their mentors and projects are listed on page 5.

Contributed by Sasha Smith

INBRE Student	Mentor	Host Institution	Project
Students from Northeastern Oklahoma State University			
Christopher Cox	Dr. David Dyer	OUHSC	*In silico* Methods to Predict Genes That Encode sRNAs in *Actinobacillus actinomycetemcomitans*
Students from Southeastern Oklahoma State University			
Josiah Schomer	Dr. Daniel Carr	OUHSC	Nervous System Expression of CXCL10 Contributes Towards Resistance to Herpes Simplex Virus Type 1 Infection
Students from Oklahoma City Community College			
David Ayadpoor	Dr. Melville Vaughan	UCO	*In vitro* Aging on Myofibroblast Phenotype
Frank Boyd	Dr. Lurdes Queimado	OUHSC	Studying a Novel Link between DNA Repair and the Wnt Pathway
Steven Craig Covey	Dr. David Dyer	OUHSC	Using Bioinformatic Techniques to Characterize Sub-Family Groups of the Fur Protein Family
Kelly Etherton	Dr. James McGinnis	OUHSC	Analyzing the Molecular Basis for the Translocation of Rod Alpha Transducin
Bao-Linh Nguyen	Dr. Michael Ihnat	OUHSC	Effect of Chronic Low Dose Arsenic and High Glucose on Hypoxic Signaling
Lauren Reeves	Dr. Mauricio Sanchez	UCO	*Abstract Title Not Available*
Clay Sandel	Dr. Michael Centola	OMRF	Evidence for IL-23 Mediated Pathology of Rheumatoid Arthritis
Jenny Stacey	Dr. Stephen Marek	OSU	Phenotypic Characterization of T-DNA Tagged Mutants of *Phoma medicaginis*
Students from Langston University			
George Kpeli	Dr. Dee Wu	OUHSC	Cisplatin Pharmacokinetic/Pharmacodynamic for Tumor Therapy: Modeling and Meta-Analysis
Contessa Majors	Dr. Muna Naash	OUHSC	The Efficacy of Compacted-DNA Nanoparticles in Ocular Gene Delivery
Students from Redlands Community College			
Taylor Arnold	Dr. Garo Philip Basmadjian	OUHSC	USP 797 End Product Verification of Commercially Available Compounded Low-Risk Radiopharmaceuticals
Colby Shepherd	Dr. Ramamurthy Mahalingam	OSU	Analysis of an Arabidopsis T-DNA Insertion Line Distriputing the DCP2 Gene
Students from Cameron University			
Linda Ash	Dr. Carla Guthridge	Cameron Univ.	Characterization of P2RX7 Expression and Function in Human Keratinocytes
Michael Landoll	Dr. Jialing Lin	OUHSC	Mutagenesis Study of the Pore-Formation by Anti-Apoptotic Bcl-2
Callie Mosiman	Dr. Sundararajan Madihally	OSU	Cell Colonization in Thermosetting Injectable Hydrogels
Sandra Pope	Dr. Leonidas Tsiokas	OUHSC	Co-Localization of TRPC1 and TRPC4 in Transfected Cells
Lora Bailey-Repp	Dr. Ira Blader	OUHSC	Oxygen Sensing in *Toxoplasma Gondii*
Valerie Toodle	Dr. Guangpu Li	OUHSC	The Specificity of TBC1D15 as a Rab GAP
Gabriel Vidal	Dr. Joel Guthridge	OMRF	Detecting Epistasis in Human Lupus: Application of Multifactor Dimentionality Reduction (MDR)
Students from Southwestern Oklahoma State University			
Samuel Cropp	Dr. Arden Aspedon	SWOSU	Osmoprotective Genes in *Pseudomonas aeruginosa*
Adarsha Koirala	Dr. Muatasem Ubeidat	SWOSU	Whole-Mount In Situ Hybridization of 5'-Nucleotidase Gene in *Dictyostelium discoideum*
Cheri Lemons	Dr. Randle Gallucci	OUHSC	Effects of Hyperglycemic Conditions on Interleukin 6 Receptor Function
Andrew Nelson	Dr. Jason Johnson	SWOSU	Mimicking Synchronization Signals in Carbamoyl Phosphate Synthetase
Anna Nelson	Dr. Doris Benbrook	OUHSC	Mitochondrial Effects of the Anti-Cancer Compound SHetA2 on Ovarian Cancer
Cammi Valdez	Dr. Han Wang	OU	Heme Deficiency Downregulates Expression of Exocrine Pancreas Genes in Zebrafish
Students from the University of Central Oklahoma			
Abdiwahab Mohamed	Dr. Wei Chen	UCO	Immunological Effect of Laser Immunotherapy in Treatment of Metastatic Tumors in Mice
Danjela Mojsilovic	Dr. Darrin Akins	OUHSC	Expression of the *E. coli* OmpA Porin in *Borrelia Burgdorferi*: The Lyme Disease Spirochete
Vagan Mushegyan	Dr. James Jarvis	OUHSC	Immunologic Function of STAT 1 in Trophoblast-Like JAR Cells
Students from Comanche Nation College			
Michael Murrow	Dr. Dennis Frisby	Cameron Univ.	Isolation of the NAG Promoter in *Caenorhabditis elegans*

The University of Oklahoma Health Sciences Center

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We're on the Web!

See us at:

microgen.ouhsc.edu

Rate structure *(partial listing; please check our web page for details)*

DNA sequencing (<96 samples)	$5/reaction	Library construction	*contact us*
DNA sequencing (≥96 samples)	$3/reaction	Human genotyping	$500/sample
DNA fragment analysis	$3/sample	Membrane array fabrication	$11/array
HLA DNA sequencing	$400/locus	Microarray scanning	$20/slide
DNA normalization, 96 samples	$10.60	Bioinformatics support	$45/hour

Bioinformatics support

We offer bioinformatics support through our Informatics Core, directed by Jeremy Zaitshik. The rate for support is $45/hour with a one-hour minimum charge. **Please note: we do not provide Tier 1 IT support.** For inquiries or work requests contact:

Jeremy Zaitshik (Jeremy-zaitshik@ouhsc.edu)
Perl programming (for sequence analysis, manipulation, etc.)
UNIX/Linux system administration
Security issues
Sequence analysis software questions
Data acquisition/automation
Database design (MySQL/PostgreSQL)
Web design (HTML/CGI)

Tim Schmidt, M.S. (Timothy-schmidt@ouhsc.edu)
Phylogenetic analysis (PAUP, PHYLIP, MEGA, PAML, etc.)
Comparative genomics (alignment, promoter analysis, etc.)
Perl programming (data manipulation, data mining, etc.)
Proteomic analysis (localization, structure, display with spdv, Rasmol, etc.)
Web design/HTML

INBRE Multicampus Bioinformatics Specialist

The INBRE MBE Specialist is responsible for fostering the development of bioinformatics education on 14 undergraduate campuses in the state of Oklahoma, and coordinating INBRE-related bioinformatics activities with the INBRE Bioinformatics Core. For inquiries and INBRE information, please contact:

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(edgar-scott@ouhsc.edu)
Bioinformatics education
Computational biology education
Sequence analysis software questions
Data acquisition/automation
Web design

About us…

The OUHSC Laboratory for Genomics and Bioinformatics is a full-service genomics facility offering DNA sequencing (small- and large-scale projects), microarray design and hybridization and other services. The Director of the Laboratory for Genomics and Bioinformatics is David Dyer, Ph.D., a Professor in the Department of Microbiology and Immunology at the University of Oklahoma Health Sciences Center. Allison Gillaspy, Ph.D., is the Associate Director of the Laboratory for Genomics and Bioinformatics and a Research Assistant Professor in the Department of Microbiology and Immunology. The University of Oklahoma is an equal opportunity institution. This publication, printed by OU Printing services, is issued by the University of Oklahoma. 150 copies have been prepared and distributed at no cost to the taxpayers of the State of Oklahoma.