Welcome to Athens!

Welcome to the first annual meeting of the SouthEastern Association of Shared Resources (SEASR, pronounced "Caesar"). This meeting provides a regional forum for core directors, managers, scientists, and technical staff to meet, discuss and collaborate on the many common issues that face us. We are an official chapter of the Association of Biomolecular Resource Facilities (ABRF). Our goal is to complement the mission of the ABRF by hosting a smaller, regional meeting.

We hope this conference will provide opportunities for you to network among life science core directors, managers and administration, to interact with colleagues, to share technical advice, and to discuss the continuing challenges associated with the operation of shared research resources and technologies. We encourage everyone to participate in the panel discussions, breakout sessions and poster sessions.

We are pleased to provide a few Travel Awards for our inaugural meeting. The travel award winners are **Jin Koh, JiQiang Yao**, **Savita Shanker**, and **Tamas Nagy**. We hope to provide this opportunity for future SEASR meetings as well.

We thank the sponsors for their support and the invited speakers, session organizers and panelists for their participation and expert advice. We also thank The Athens Convention Visitors Bureau and Nashville Convention & Visitors Corporation for the use of the front and back cover photos. Special thanks go to the ABRF executive board for their continued support.

We look forward to a productive and interesting meeting!

Sincerely,

The SEASR 2013 Executive Committee:

President: David Blum, University of Georgia

Treasurer: Tracy Cooper, Vanderbilt University

Secretary: Connie Nicklin, University of Florida ICBR

Melinda Sellevold, University of Georgia

Myriam Belanger, University of Georgia

Rob Carnahan, Vanderbilt University

Travis Glenn, University of Georgia

Table of Contents

Meeting Schedule

Thursday, June 13, 2013			
Starts	**Ends**	**Event**	**Location**
4:00 PM	5:00 PM	**Registration Open**	Next to Masters Hall
6:00 PM	9:00 PM	**Opening Reception at the Foundry** Complimentary shuttle between UGA and The Foundry every 30 minutes from 5:45-9:15 PM	Pick up shuttle on corner of Carlton and S Lumpkin St
Friday, June 14, 2013			
Starts	**Ends**	**Event**	**Location**
7:30 AM	8:30 AM	**Registration Open**	Next to Masters Hall
7:30 AM	8:30 AM	**Breakfast (included)**	Magnolia Ballroom
8:15 AM	8:30 AM	**Opening Remarks** **David Blum**, SEASR President and Director, Bioexpression and Fermentation Facility, University of Georgia	Magnolia Ballroom
8:30 AM	9:00 AM	**Welcome from ABRF** **David Friedman**, ABRF President	Masters Hall
9:00 AM	10:00 AM	**Plenary Session** **Shawn Levy**, Hudson Alpha Institute "Genomics of the 21st Century; a thirteen-year review of successes, failures, and what lies ahead"	Masters Hall
10:00 AM	10:30 AM	**Coffee Break (light snacks)**	Pecan Tree Galleria
10:00 AM	8:00 PM	**Exhibit Hall Open**	Pecan Tree Galleria
10:30 AM	11:30 AM	**Panel Discussion: Metrics to Success** **John Wunderlich**, Director of Core Facility Business Services, University of Georgia **Karen DeMeester**, Director of Program Evaluation Group, University of Georgia **Ken Drescher**, Managing Director at Six Sigma Performance Improvement, Inc. **Robert Vandenberg**, Chaired Professor, Department of Management, Terry College of Business, University of Georgia	Masters Hall
11:30 AM	12:45 PM	**Lunch (included)**	Magnolia Ballroom

Friday, June 14, 2013			
Starts	**Ends**	**Event**	**Location**
12:45 PM	1:45 PM	**Vendor Workshop: Science Exchange** "Best Practices for Marketing, Payment, and Management of Core Facilities" **Bilal Mahmood**, Head of Customer Development	V
		Vendor Workshop: Illumina "Putting Data and Analysis in the Hands of Your Customers" **Michael Janis**, Product Manager	YZ
		Vendor Workshop: ForteBio Tech Talk "BLI Studies of Anthrax Toxin and Development of Diagnostic Antibodies" **Jason M. Goldstein**, Ph.D., Team Leader Immunochemistry and Cellular Development, Centers for Disease Control and Prevention	U
1:45 PM	2:00 PM	**Break (beverage service)**	Pecan Tree Galleria
2:00 PM	3:00 PM	**Vendor Workshop: Affymetrix** "The Human Transcriptome 2.0 Array & Transcriptome Analysis Console: Getting the Most Comprehensive View of Biology With No Assembly Required" **Gianfranco de Feo**, Ph.D., Sr. Director, Regional Marketing - North America	V
		Vendor Workshop: Illumina "Illumina's Sample Prep Overview" **Omayma Al-Awar**, Manager, Sales Specialist	YZ
		Vendor Workshop: HTGMolecular "A Simple System for Measuring Gene Expression in FFPE Tissue Specimens" **Chris Roberts**, VP Marketing and Medical Innovation	U
		Vendor Workshops: Life Technologies "Next-Generation Sequencing Innovations and Applications with Ion Torrent™ Technology" **Jeff Barnes**, Ph.D., Technical Sales Specialist "Digital PCR Made Simple – Hands-on QuantStudio™ 3D Digital PCR System Workshop" **Dave Chappell**, Ph.D., Field Applications Scientist	T

Friday, June 14, 2013			
Starts	**Ends**	**Event**	**Location**
3:00 PM	3:30 PM	**Coffee Break (light snacks)**	Pecan Tree Galleria
3:30 PM	4:30 PM	**Plenary Session** **Mark Lively**, Wake Forest School of Medicine "Core Laboratories in a Challenging Funding Environment"	Masters Hall
4:30 PM	4:45 PM	**Short Break**	Pecan Tree Galleria
4:45 PM	5:45 PM	**Panel Discussion: Effectively Communicating in the World of Shared Resources** **Andrew Vinard**, Manager of Biotechnology Resources, Miller School of Medicine, University of Miami **Sige Burden**, Senior Director, Faculty & Staff Relations, University of Georgia **Josh Rosenberg**, Director of Cost Studies, Emory University **T. "Soma" Somasundaram**, Director of the Macromolecular X-ray Diffraction and Crystallization Lab, Florida State University	Masters Hall
6:00 PM	8:00 PM	**Poster Session and Dinner Reception (Taste of Italy)**	Pecan Tree Galleria

Saturday, June 15, 2013			
Starts	**Ends**	**Event**	**Location**
7:30 AM	8:30 AM	**Breakfast (included)**	Dogwood Hall
7:30 AM	11:15 AM	**Exhibit Hall Open**	Pecan Tree Galleria
8:30 AM	9:30 AM	**Plenary Session** **Steve Briggs,** University of California, San Diego "Reconstruction of Protein Networks from an Atlas of Maize Proteotypes"	Masters Hall
9:30 AM	10:00 AM	**Coffee Break (light snacks)**	Pecan Tree Galleria
10:00 AM	11:00 AM	**Breakout Session: Flow Cytometry** **Julie Nelson**, University of Georgia	VW
		Breakout Session: qPCR and Microarray **Sharon Norton** and **Yanping Zhang**, University of Florida ICBR	TU
		Breakout Session: Administration **Connie Nicklin**, University of Florida ICBR	YZ
11:00 AM	11:15 AM	**Break (beverage service)**	Pecan Tree Galleria
11:15 AM	12:15 PM	**Breakout Session: Cell Imaging** **Adam Marcus**, Emory University	VW
		Breakout Session: Genomics **Myriam Belanger**, University of Georgia	TU
		Breakout Session: Antibody Technology **Dennis Bagarozzi**, Centers for Disease Control and Prevention	YZ
12:15 PM	1:30 PM	**Lunch (included)**	Dogwood Hall
12:30 PM	1:00 PM	**Closing Meeting**	Dogwood Hall

Speaker Abstracts

Genomics of the 21st Century; a Thirteen-Year Review of Successes, Failures, and What Lies Ahead

Shawn Levy

HudsonAlpha Institute for Biotechnology, Huntsville, AL

The wide-spread availability of genomic technologies and development of core facilities supporting them began in the late 90's and really picked up momentum over the last thirteen years. The unprecedented pace of technology evolution, application development and data volume has generated challenges for core facility management and success that have been both unusual as well as enabling. This presentation will discuss the successes and failures over the last 13 years of operating large-scale genomic core facilities at both small and large institutions performing both simple as well as complex projects in collaboration with outstanding laboratories and investigators from around the world.

Core Laboratories in a Challenging Funding Environment

Mark O. Lively

Wake Forest School of Medicine, Winston-Salem, NC

This presentation will focus on the history of core laboratory evolution in academic research institutions and on the impact that the current science funding climate is having on such labs. Dr. Lively has operated a shared resource laboratory for protein and DNA chemistry since the early 1980s. He is a founding member and former president of the Association for Biomolecular Resource Facilities (ABRF). In addition to his experiences as a funded investigator and core laboratory director, Dr. Lively has also been very involved in advocacy for science policy and funding at the national level, in Congress and at the National Institutes of Health and the National Science Foundation. He has been a member of the board of directors of the Federation of American Societies for Experimental Biology (FASEB) and served as its President (2009-2010). He was a member of the final national advisory council for the National Center for Research Resources at the NIH and is a current member of the NIH Council of Councils that directly advises the NIH Director, Dr. Francis Collins. Dr. Lively will discuss the impact of the budget sequestration process on the NIH. As a core laboratory director, principal investigator, and science advisor, Dr. Lively is able to offer a broad perspective on the critically important role of shared resource facilities in enhancing the effort of biomedical scientists.

Reconstruction of Protein Networks from an Atlas of Maize Proteotypes

Steven Briggs

UCSD Division of Biological Sciences, San Diego, CA

A comprehensive knowledge of proteomic states is essential for understanding biological systems. Using mass spectrometry, we mapped an atlas of developing maize seed proteotypes comprising 14,165 proteins and 18,405 phosphopeptides (from 4,511 proteins), quantified across eight tissues. We found that many of the most abundant proteins were not associated with cognate mRNAs. Further, protein abundance was poorly correlated with mRNA levels and was largely independent of phosphorylation status. Comparisons between proteotypes revealed the quantitative contribution of specific proteins and phosphorylation events to the spatiotemporally regulated starch and oil biosynthetic pathways. Reconstruction of signaling networks revealed associations of proteins and phosphoproteins with distinct biological processes acting during seed development. Additionally, a protein kinase-substrate network was reconstructed enabling the identification of potential substrates of specific protein kinases. Finally, we observed enrichment of transcription factor families and their phosphorylated forms in specific seed tissues.

Panel Discussions

Metrics to Success

Moderator

John Wunderlich, Director of Core Facility Business Services, University of Georgia

Panelists

Karen DeMeester, Director of Program Evaluation Group, University of Georgia

Ken Drescher, Managing Director at Six Sigma Performance Improvement, Inc.

Robert Vandenberg, Chaired Professor, Department of Management, Terry College of Business, University of Georgia

Abstract

What is success and how do you get there? This panel will first discuss how core facilities can determine the elements that are important for them to be considered successful. Then we will discuss how to identify measurements to gauge progress towards improvement.

Effectively Communicating in the World of Shared Resources

Moderator

Andrew Vinard, Manager of Biotechnology Resources, Miller School of Medicine, University of Miami

Panelists

Sige Burden, Senior Director, Faculty & Staff Relations, University of Georgia

Josh Rosenberg, Director of Cost Studies, Emory University

T. "Soma" Somasundaram, Director of the Macromolecular X-ray Diffraction and Crystallization Lab, Florida State University

Abstract

In the realm of Core Facilities and Shared Resources, many individuals play a role in realizing success. Unfortunately, the many individuals often come from varied backgrounds and as such may have differing languages. Core Directors and technicians speak science and product, administrators speak administration, clients speak project specific science, and leadership speaks bottom line. This panel will seek to contextualize the problem further, and provide some guidance on how to make your cores communicate effectively to thrive in an era of shrinking resources.

Agenda

Opening	Andrew Vinard	
Strategic communication	Josh Rosenberg	Identifying stakeholders, and laying out a strategy for communication
Methods of communication	T. "Soma" Somasundaram	From emailing to holding user meetings, how to speak with your stakeholders
Conflict Resolution	Sige Burden	What to do when you don't see eye to eye with your stakeholders
Discussion		

Poster Abstracts

1

Establishing New Services and Enhancing Project Management at the ICBR Gene Expression Core

Yanping Zhang, David Moraga, William Farmerie and Robert Ferl

Interdisciplinary Center for Biotechnology Research (ICBR), University of Florida, Gainesville, FL

Our mission is to provide state-of-the-art technical services and consultation on Gene Expression technology to researchers at the University of Florida, and throughout the state and nation. The leading edge of technology constantly advances. Keeping core services at the technological forefront can be challenging. The ICBR Gene Expression Core (GE) provides a variety of full services including microarray gene expression profiling and constructing RNA-Seq libraries. Unexpected opportunities leading to new services may arise by working with outside groups and vendors. Managing projects also carries responsibility for timely client communications and their project moving toward on time completion. We describe two recent experiences developing new services and present our formalized process for client communication.

We participated in the ABRF Next Generation Sequencing Research Group study (ABRF-NGS). The ABRF-NGS study brought us together with other ABRF members and Pacific Biosciences scientists to develop new methodology for transcriptome analysis. We focused on full-length cDNA sequencing of mature, complete transcripts by exploiting the extraordinary long read length capabilities of our Pacific Biosciences RS instrument.

Acquiring new instrumentation with advanced capabilities is another challenge faced by most core facilities. By partnering with the Office of the UF Vice President for Research and the UF College of Veterinary Medicine, we acquired a GenePix 4-laser scanner as part of a package needed to recruit an exceptional faculty member. By taking a broader institutional view of new instrument acquisition and placing the GenePix in the ICBR GE Core where it benefits all UF scientists, makes new technology available, benefits faculty recruitment, and helps us establish new services.

Achieving high-level project management skills is a challenge for most core facilities. By implementing a formalized process called SMART Project Management (SPM), we enhanced our service delivery, created a better client relationship and enabled a simple and efficient project tracking system.

2

High Affinity Peptides to *Bacillus anthracis* Lethal Factor mAb by Phage Display Selection

Jason M. Goldstein[1], Dennis A. Bagarozzi,Jr.[1], Uzma Ansari[1], Amanda Lyons[1], Curtis Taylor[1], Anne Boyer[2] and Conrad P. Quinn[3]

[1]Immunochemistry and Cellular Development Team/Scientific Products and Support Branch//Division of Scientific Resources/NCEZID

[2]Clinical Chemistry Branch/DLS/NCEH

[3]Meningitis and Vaccine Preventable Diseases Branch/MVPD/Division of Bacterial Diseases/NCIRD

Peptides which bind to a *B. anthracis* anti-Lethal Factor (LF) monoclonal antibody (mAb) were selected from a random phage-display peptide library to elucidate the mechanism for mAb-stimulated catalytic activation of LF protease. The library was surface panned followed by individual clone selection and sequences evaluation. Peptides from phage clones were found to interact at low nM affinity with the diagnostic mAb. LF recombinant protein could competitively reduce the interaction of phage peptide with the antibody. Discovered sequences provided a consensus which defines a continuous epitope (THQDEI) within a structural loop implicated in mechanisms of LF substrate recognition.

3*

Sequencing of Structurally Complex DNA Regions with Sanger and PacBio Technologies

Savita Shanker, N.G.. Panayotova, X.H. Zhou, S. Hampton, D. Moraga, and W.G. Farmerie

Genomics Division of the Interdisciplinary Center of Biotechnology Research, University of Florida, Gainesville, FL

Sanger sequencing remains a valuable tool for determining nucleotide sequences of individual DNA molecules. For large genome projects, Next-Gen sequencing technologies have supplanted Sanger sequencing because of its scalability, speed and diminished overall cost. However, Sanger sequencing is still widely used for small-scale projects and for obtaining contiguous reads of 800-1000bp. Although, Sanger sequencing is considered as the gold standard for sequencing, in many cases it fails to give high quality reads through complex DNA regions e.g. DNA with high GC rich content, complex sequence repeats, hairpin structures and long homopolymer stretches. These limitations have not been overcome by second generation sequencing technologies. PacBio, the third generation sequencing technology provides an approach that is different from Sanger and second generation sequencing technologies, and has potential to overcome common DNA sequencing challenges by providing significantly longer reads (average readlength >4,000bp). Here, we report nucleotide sequence data for a plasmid template containing inverted tandem repeats with long Poly G and Poly C and regions with strong hair pin structures using a specially modified Sanger sequencing approach and with PacBio sequencing.

*** Denotes SEASR 2013 Travel Award Recipient**

4*

A Novel Tool for Massive Parallel Pyrosequencing Data Analysis of IgG Region

JiQiang Yao[1], L. Yin[2], K.Chang[2], J. Sleasman[3], FH. Yu[1] and M. Goodenow[2]

[1]Interdisciplinary Center for Biotechnology Research, University of Florida, Gainesville, FL

[2]Dept. of Pathology, Immunology and Laboratory Medicine, University of Florida College of Medicine, Gainesville, FL

[3]Cancer Epidemiology, H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL

Complementarity determining regions (CDRs) are hypervariable regions of immunoglobulins. CDRs determine antibody specificity and affinity for specific antigens. Within the variable domain, CDR3 is the most variable as it is determined by combinatorial V-D-J (heavy chain) or V-J (light chain) rearrangements, P and N-addition, junctional flexibility, and somatic hypermutation of variable domain nucleotides. The 454 high-throughput pyrosequencing technologies have generated long enough sequences to span CDRs of a chain in a single read. It's possible to capture the diversity and characterize the number of unique binding surfaces presented by CDR3 region. Nevertheless, the unprecedented volume of sequencing data creates a great challenge in data analysis. In this study, we present a novel bioinformatics tool (Igseq) that can automate the data analysis process. It accepts IgG sequence data and makes the following analysis: somatic hypermutation, gene allele usage, metagenomics analysis of antibody diversity and antibody selection analysis under antigen stimulation. This pipeline integrates the international ImMunoGeneTics information system IMGT® as its core for junction prediction. It has the following functions: i) Mutation Analysis: Frequency of nucleotide and amino acid mutation, sequence hydropathy, net electric charge. ii) Metagenomics analysis: Antibody repertoire was analyzed using ESPRIT. Estimation of species richness (ACE) was made by the rarefaction curves. iii) Antigen Selection Analysis: Under antigen stimulation, the IgG sequences with higher replacement mutations than random mutation were identified with 95% confidence. The software was tested with 850,762 pyrosequences of 57 samples showed that the software is fast and reliable. Only 1G memory is required and each sequence takes one second. The analysis results are presented both in data and graph formats.

*** Denotes SEASR 2013 Travel Award Recipient**

5

Genotyping and Fragment Analysis Services at the University of Florida's ICBR Genotyping Core Laboratory

Clark, A.M. and A. Gomez

Genotyping Division of the Interdisciplinary Center of Biotechnology Research, University of Florida, Gainesville, FL

The Interdisciplinary Center for Biotechnology Research's (ICBR) Genotyping Facility (GT) at the University of Florida is a core facility dedicated to identifying and analyzing PCR fragments, SNPs, and gene expression assays. Our state of the art instrumentation allows us to offer researchers services at a reasonable fee. GT employs cutting edge technology to design de novo microsatellite libraries for non-model species such as ferns, burrowing owls, and rattlesnakes. Custom (ie, eucalyptus, apples, citrus) and off the shelf Illumina SNP and gene expression platforms are processed on our BeadArray Reader and traditional fragments generated by PCR are analyzed on either the AB3730xl or the Advance FS96 instruments. The GT staff strives to produce the best quality data available in a timely manner. Let us help you advance your innovative project by providing excellent service and data.

6

New and highly effective automated solutions for sample preparation for next-generation sequencing

Josh Mann

QIAGEN Inc., Germantown, MD

Next-generation sequencing (NGS) requires specialized and often time-consuming methods to select particular nucleic acid fractions and generate libraries suitable for sequencing. We present new, automatable methods to deplete ribosomal RNA (rRNA) from a total RNA sample for sequencing and high-yield library construction.

7

Proteomics and Mass Spectrometry Applications in Biological and Medical Research

Jin Koh, Jinxi Li, Marjorie Chow, Ran Zheng, Cecilia Silva-Sanchez, Sixue Chen

Proteomics Division, Interdisciplinary Center for Biotechnology Research, University of Florida, Gainesville, FL

Proteomics and mass spectrometry have provided unprecedented tools for fast, accurate, high throughput biomolecular separation and characterization, which are indispensable towards understanding the biological and medical systems. Studying at the protein level allows researchers to investigate how proteins, their dynamics and modifications affect cellular processes and how cellular processes and the environment affect proteins. The mission of our facility is to provide excellent service and training in proteomics and mass spectrometry to UF scientists and students. Here we present our capabilities in proteomics and other analytical services. The tools include a gel-based 2D-DIGE (Two Dimensional Difference Gel Electrophoresis), shotgun sequencing, and gel-free iTRAQ (Isobaric Tags for Relative and Absolute Quantitation). Along with our capacity of separating thousands of proteins and characterizing differential protein expression, we have a suite of state-of-the-art mass spectrometers available for biomedical sciences and advanced technology research, including a tandem time-of-flight (4700 Proteomics Analyzer, AB), quadrupole/time-of-flight (QSTAR XL and Elite, AB), hybrid quadrupole-linear ion-trap (4000 QTRAP, AB), and LTQ-Orbitrap FTMS. These instruments are mainly used for protein identification, posttranslational modification characterization and protein expression analysis (e.g., Mass Western). Our facility is also set up to provide Biacore biomolecule interaction analysis. Proteomics and mass spectrometry are useful in large-scale survey of proteome for hypothesis generation as well as in detailed analysis of target proteins for hypothesis testing. Our services also include accurate molecular weight analysis, MRM-based protein screening and targeted metabolite profiling. To ensure success and maximize productivity, the facility offers education, consultation, data processing and reporting, and support of grant application.

8*

De novo transcriptome Assembly and Proteome Profiling of the Recently Formed Allopolyploid *Tragopogon mirus* (Asteraceae) and its Diploid Parents

Jin Koh[1], Mi-Jeong Yoo[2], Douglas Soltis[2,3,4], Pamela Soltis[3,4], William Barbazuk[2], Lauren McIntyre[5], Sixue Chen[1,2,3]

[1]Interdisciplinary Center for Biotechnology Research, University of Florida, Gainesville, FL

[2]Department of Biology, University of Florida, Gainesville, FL

[3]Genetics Institute, University of Florida, Gainesville, FL

[4]Florida Museum of Natural History, University of Florida, Gainesville, FL

[5]Department of Molecular Genetics and Microbiology, University of Florida, Gainesville, FL

Polyploidy (whole-genome duplication) is recognized as an important evolutionary process in speciation and genome evolution of diverse organisms, particularly plants. However, much of our current understanding of polyploidy is based on analyses of crop species. Here, we examined the transcriptomes of naturally occurring, recently formed, allopolyploid *Tragopogon mirus* and its diploid parents (*T. dubius* and *T. porrifolius*) using Illumina HiSeq 2000 technology. In parallel, we employed iTRAQ LC MS/MS to investigate the global proteomes of the three species. A total of 480 million 100-bp paired-end reads was generated from leaf transcriptomes of the three species, which corresponds to 34 Gb of sequence. These reads were assembled *de novo* by the Trinity short-read assembler, and this assembly was utilized as a reference for RNA-Seq and proteomic data analyses. Differential gene expression between the allopolyploid and its diploid parents was analyzed and compared at transcriptomic and proteomic levels. Together with ongoing analysis of transcriptome profiles of another young allopolyploid, *T. miscellus*, and its diploid parents (*T. dubius* and *T. pratensis*), this study will provide valuable insights into transcriptomic as well as proteomic changes in recently formed allopolyploids. In addition, the transcriptome data set generated here provides the most comprehensive sequence resource for the *Tragopogon* polyploid system, which enables further studies of gene and protein expression patterns in different tissues and under different conditions.

*** Denotes SEASR 2013 Travel Award Recipient**

9*

Phenotyping Challenges in Genetically Engineered Mice

Tamas Nagy

Department of Pathology, College of Veterinary Medicine, University of Georgia, Athens, GA

Genetically engineered mice (GEM) are a staple of modern biomedical research. While genetic engineering technology has become more and more sophisticated, proper pathological analysis of these animals is often lacking. Proper pathological analysis is often replaced by Do-it-Yourself (DIY) pathology, which often resulted in surprising "scientific discoveries". This study's objective was to highlight published instances of erroneous interpretations of lesions and non-lesions in genetically engineered mice in the hope that more biomedical investigators can be persuaded to involve properly trained and experienced veterinary pathologists in their evaluation of newly created GEM. The main reasons of erroneous interpretation were found to be either unfamiliarity with mouse anatomy and/or histology or misinterpretation of inflammatory or proliferative in lesions in mice as neoplastic processes. Proposed solutions to this acute problem are presented as well. First, training institutions should modify their training program to include instruction in mouse and GEM pathology. Second, scientific peer review of manuscripts on GEM should involve an appropriately credentialed veterinary pathologist. Third, funding agencies should recruit veterinary pathologists to their study sections to evaluate proposals on GEM.

*** Denotes SEASR 2013 Travel Award Recipient**

10

Comparison of qNPA vs qPCR on FFPE Tissue

Chris Roberts[1], Matt Rounseville[1], Klaus Pechhold[2], Debrah Thompson[1], Mark Schwartz[1], Bruce Seligmann[3]

[1]HTG Molecular Diagnostics, Tucson, AZ

[2]University of Massachusetts Medical School, Worcester, MA

[3]MDDx, Tucson, AZ

Accurate, sensitive and robust multiplexed measurements of gene expression from formalin fixed paraffin embedded (FFPE) tissue or paraformaldehyde fixed samples are ideally required for clinical diagnostic tests and retrospective analysis of archived samples. We evaluated the measurement of gene expression from FFPE tissues using HTG Molecular's qNPA™ assay and made comparisons to qPCR. The results show that qNPA provides a highly sensitive, accurate, quantitative, and robustly reliable automated multiplexed assay of gene expression from FFPE, permitting the rapid development and launch of new diagnostic and research assays. qNPA uses a lysis-only, extraction-free protocol which measures up to 47 genes/sample. In contrast, qPCR requires RNA extraction/reverse transcription. Accuracy was determined by correlating measurements from matched frozen and fixed samples, determining the R2 correlation coefficient. Comparing frozen/FFPE cell pellets qNPA R2 = 0.97. Comparing frozen/FFPE pancreas tissue qNPA R2= 0.97 with a present call rate of 91%, compared to qPCR R2= 0.86 and a present call rate of just 17%, even using 26 times more FFPE sample amount than qNPA. Comparing fresh/paraformaldehyde fixed Islet cells, qNPA R2= 0.98, qPCR R2= 0.02, after staining for sorting by cytometry qNPA R2= 0.96, qPCR R2= 0.12. Thus, qNPA fixed tissue measurements were much more accurate than qPCR. In an additional comparison using matched breast cancer frozen compared with FFPE samples, qNPA measurements averaged R2=0.81, with 6% CV for triplicate measurements of separately processed samples. For >95% of genes the expression levels measured by qNPA were independent of cold ischemic times of 0 to 16 hr. Measured levels are also independent of fixation from 4 to 72 hr. The robust performance of qNPA measurements from FFPE translated into quantitative consistency that has previously not been achievable using qPCR. Data from clinical FFPE samples submitted by a large number of HTG's clients was reviewed and for each set the number of samples tested, the average area tested/sample (cm2 area of a 5 micron thick section), the failure rate (FR), and the avg reproducibility (%CV for samples independently processed in triplicate) was determined for breast FFPE (300 samples, 0.3 cm2/sample, 1% FR, 10.5%CV), lung FFPE (700 samples, 0.3 cm2/sample, 0.5% FR, 9.5%CV), prostate FFPE (15 samples, 0.3 cm2/sample, 0% FR, 11%CV), colon FFPE (15 samples, 0.3 cm2/sample, 0% FR, 9%CV), ovary FFPE (15 samples, 0.3 cm2/sample, 0% FR, 10%CV) and lymphoma FFPE (300 samples, 0.3 cm2/sample, 0.5% FR, 8%CV). Thus, qNPA provides a highly accurate, reproducible, robust multiplexed measurement of gene expression from FFPE tissue that is not affected by wide variations in ischemic or fixation time. Fully automated qNPA on the EDGE platform is expected in Jan 2013. This system, with a turn-around time of less than 24 hours, will improve even further the robustness of extraction-free gene expression.

11

Research Infrastructure Improvement Via Core Consolidation

William Roth, P. Alexander, L. Anderson, M. Bacanamwo, N. Emmett, M.B. Huang, J.W. Lillard, K. Lo, D. Lyn, D.V. Parker, J. Patrickson, M.D. Powell, Q. Song, X. Yao, and VC Bond

Research Centers in Minority Institutions Program, Morehouse School of Medicine, Atlanta GA

Purpose: The research cores and shared-use facilities at Morehouse School of Medicine (MSM) were initially established with NCRR/RCMI funding and are available to all MSM researchers. These facilities and research infrastructure services have been essential in: 1) attracting well-qualified faculty recruits, 2) supporting existing research initiatives, and 3) establishing and expanding graduate programs at MSM. Optimization of the cores is necessary to allow MSM to continue its positive trajectory for research productivity. A key part of future plans for RCMI-funded core facilities is consolidation within a single physical area.

Design Methods: A design committee was formed to address core consolidation under the following aims: 1) Provide a physical environment that promotes optimal core efficiency and productivity, optimizing shared equipment and human technical resources. 2) Provide a collegial core environment that enhances inter-unit collaboration at all levels, leading to scientific interchange and collaboration among investigators and enhanced research training. 3) Provide research infrastructure necessary for state-of-the-art research in strategic research focus areas.

Results: Detailed plans were drawn up, placing the main research cores in an open-lab configuration. An application was submitted to NIH in response to RFA-RR-09-007 [Core Facility Renovation, Repair, and Improvement (G20)]. Notification of award was received in 2010.

Conclusion: At the project's end in 2013, the consolidated RCMI core space will contain both research core labs and space for administrative and support services. The re-location of research labs and services will support ongoing improvement of the research infrastructure at MSM. GRANT SUPPORT: NIH/NCRR/RCMI G12-RR03034.

12

Novel Clinical & Translational Methods, Technologies & Resources

Andrew Vinard and Tamara Levine

Miller School of Medicine, University of Miami, Miami, FL

Information on the University of Miami's (UM) core facilities in context of our Clinical and Translational Science Institute (CTSI). The purpose of the UM CTSI is to provide a comprehensive platform that fosters an interdisciplinary academic environment in which researchers are inspired and fully supported in their efforts to develop new lines of investigation and novel scientific methods.

Specific Aims include: 1) Facilitate access and utilization of core resources, identify need for new cores, 2) Accelerate the development of novel clinical and translational methods by promoting interdisciplinary research.,3) Provide a mechanism to link studies with commercial potential to technology transfer resources, and 4) Track development of CTSI-sponsored novel methods and address incorporating multidisciplinary work into tenure track system.

Additional information on transforming health research at UM, evaluation and milestones, and work in progress is also presented.

13

RNA Isolation: Developing a New Core Laboratory Service for Internal Workflow Enhancement and Expansion of External Client Offerings

Sharon Norton[1], Andre Clark[1], David Moraga[1], Scott Jamison[2]

[1]Genomics, Interdisciplinary Center for Biotechnology Research, University of Florida, Gainesville, FL

[2]Life Technologies Corporation, Grand Island, NY

RNA isolation is an integral step in many of the protocols associated with gene function and expression such as Northern blotting, in vitro translation, cDNA library construction, microarray, qPCR, and RNASeq. Many of these services are provided in a core laboratory and require the highest quality microRNA, total RNA or messenger RNA input to have a successful experimental outcome. With the huge variety of organisms and strategies for RNA isolation, and just as variable pricing for the service itself, careful background research and consideration is crucial to implement this technique as a new core laboratory service offering. It is suggested that RNA isolation services require close cooperation with users to ensure that their sample collection protocols are suited for good quality RNA output. When offered in a Core lab setting, RNA isolation services offer the additional, potential advantage of coupling the service with other downstream Core services that the user may require. Such collaboration affords a better handle and control on the quality of the input material for RNA downstream applications, while also aiding in revenue-generating activities for the Core facility. The information presented here is a summary of the steps taken to decide how to implement the new service RNA isolation as a supporting function to other ICBR Core laboratories and offer the general UF researcher population this option for their research needs.

14

Translating microRNA Discovery in Biofluids into Robust Biomarkers for Disease Using LNA™-Enhanced qPCR

Brian Glassner, Peter Mouritzen, Thorarinn Blondal, Ditte Andreasen, Niels Tolstrup, Maria Wrang Teilum

Exiqon, Woburn, MA

microRNAs represent the best described class of small RNAs (21-23nt) and have been shown to function as post-transcriptional regulators of gene expression. The high relative stability of microRNA in common clinical source materials and the ability of microRNA expression profiles to accurately classify discrete tissue types and specific disease states have positioned microRNA quantification as a promising new biomarker for a wide range of diagnostic applications.

We have developed a genome-wide LNA™-based microRNA qPCR platform with unparalleled sensitivity and robustness even in biofluids where microRNA levels are extremely low. Only a single cDNA synthesis reaction is required to conduct full miRNome profiling thereby facilitating high-throughput profiling in important clinical sources without the need for pre-amplification. Thousands of biofluid samples have been profiled including blood derived plasma/serum and urine to accurately determine normal reference ranges for circulating microRNAs. Procedures have been developed to control pre-analytical variables such as hemolysis in serum/plasma samples. In addition, a data QC system has been implemented to secure technical excellence and reveal any unwanted bias in the dataset.

We are currently screening for and validating microRNAs as biomarkers for stage II colorectal cancer (CRC). microRNA profiling has been performed on plasma samples from a clinical trial conducted in 7 different hospitals. We show that hemolysis in this sample set correlates with hospital ID, and with the utilization of specific blood sample collection vials. Using a microRNA-based hemolysis signature, we eliminated hemolyzed samples and demonstrated that this step leads to a major improvement of CRC detection (ROC AUC increase from 0.67 to >0.80). We conclude that pre-analytical variables such as hemolysis can be a source of bias in samples of different origin, and that sample and data QC procedures can overcome this challenge and lead to improved miRNA biomarker performance.

15

Co-optimization of Probes and Polymerases to Drive the Evolution of Targeted Sequencing

James Furbee

Roche Diagnostics Corporation, Indianapolis, Indiana

The evolution of targeted enrichment methods for high-throughput sequencing applications has focused optimization efforts onto a small number of persistent technical impediments to increased throughput and performance. Primarily, these include inefficiencies in library construction, automation-unfriendly workflows, and the well-documented tendency of many DNA polymerases to introduce strong biases against AT- and GC-rich targets. By combining enzymes specifically tailored for high performance NGS applications with innovative oligonucleotide probe design, we developed protocols for improved library construction and targeted enrichment. The result is a workflow that retains input sample complexity, minimizes amplification biases and artifacts such as PCR duplicates and chimeric library inserts, is compatible with manual or high-throughput workflows, and delivers unparalleled sensitivity for variant discovery over a wider range of targets throughout the genome than had previously been demonstrated.

We applied the new reagent and protocol combinations to a series of enrichment targets, including human exomes and panels of genomic targets specifically designed to challenge capture performance with (A+T)-rich and (G+C)-rich targets. Sequencing of enriched material was performed on the Illumina HiSeq instrument. The results presented here extend the capabilities of targeted sequence enrichment, a method that has already transformed the work of genome analysis, and will enable the future discovery of more variation, in more regions of the genome, in more samples, and in less time.

16

Long Jump: High Diversity Mate-Pair Libraries from Sub-Microgram Amounts of Genomic DNA

Matthew Hims, Ole Schulz-Trieglaff, Helen Bignell, Niall Gormley and Geoffrey Smith

Illumina Cambridge Ltd, Saffron Walden, UK

For the purpose of shotgun de novo genome assembly it was recognized and demonstrated that sequencing distant ends of a long DNA molecule provides significant benefits for improving the continuity of the assembly. Generating such mate-pair libraries via limited mechanical shearing, ligation of biotinylated adapters, circulation and paired-end sequencing of the biotin purified smaller DNA fragments remained technically challenging and inefficient until recent advances in sample preparation. Here we are presenting two updated mate-pair library protocols that employ a modified Nextera tagmentation workflow for simultaneous DNA fragmentation and addition of biotinylated adapters that may or may not be followed up by size selection. Besides simplifying and shortening the workflow other improvements include a reduction in DNA input requirements and an increase in library diversity in the absence of size selection. The functional utility of the Nextera mate-pair library protocol is demonstrated by comparing de novo assemblies of various bacterial genomes into scaffolds containing significantly fewer gaps.

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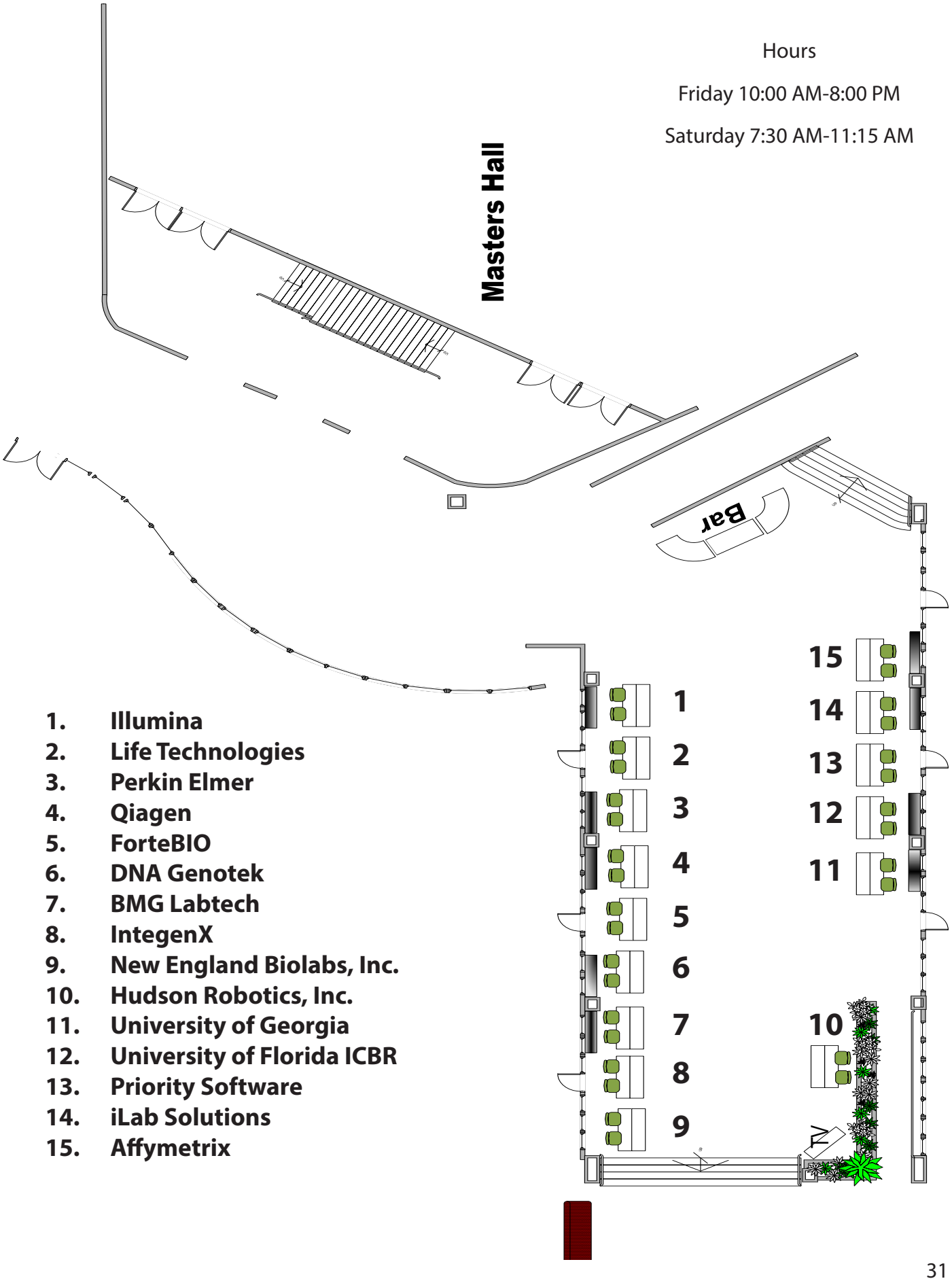

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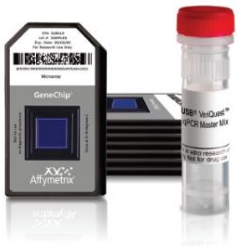

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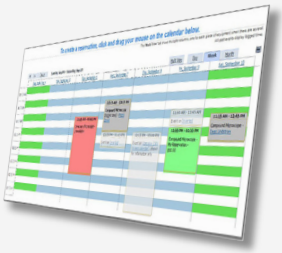

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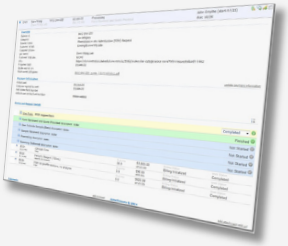

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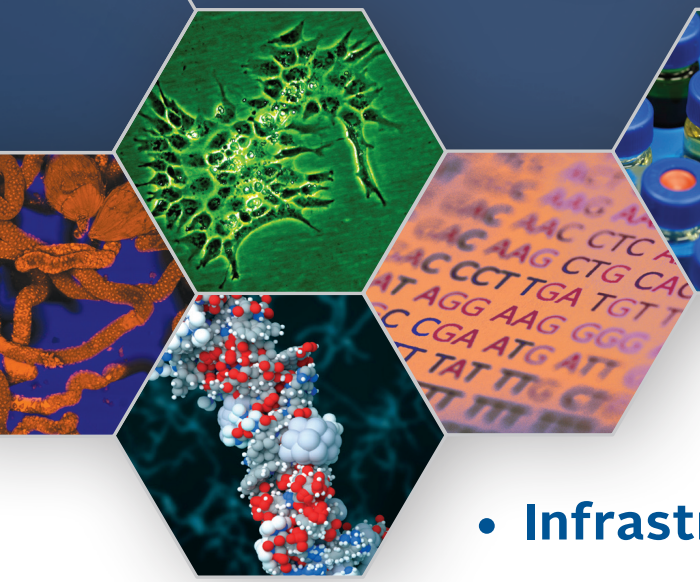

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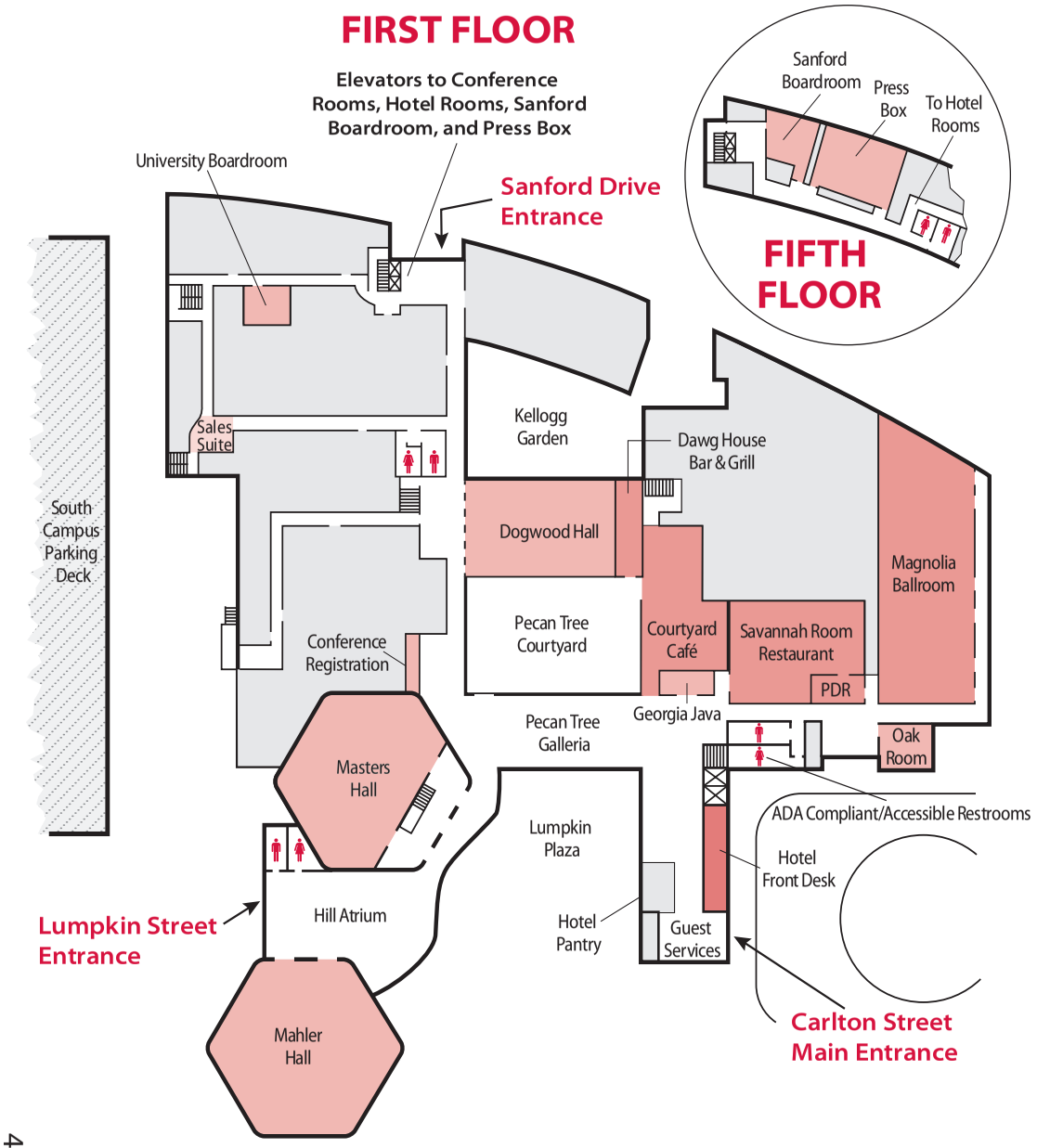

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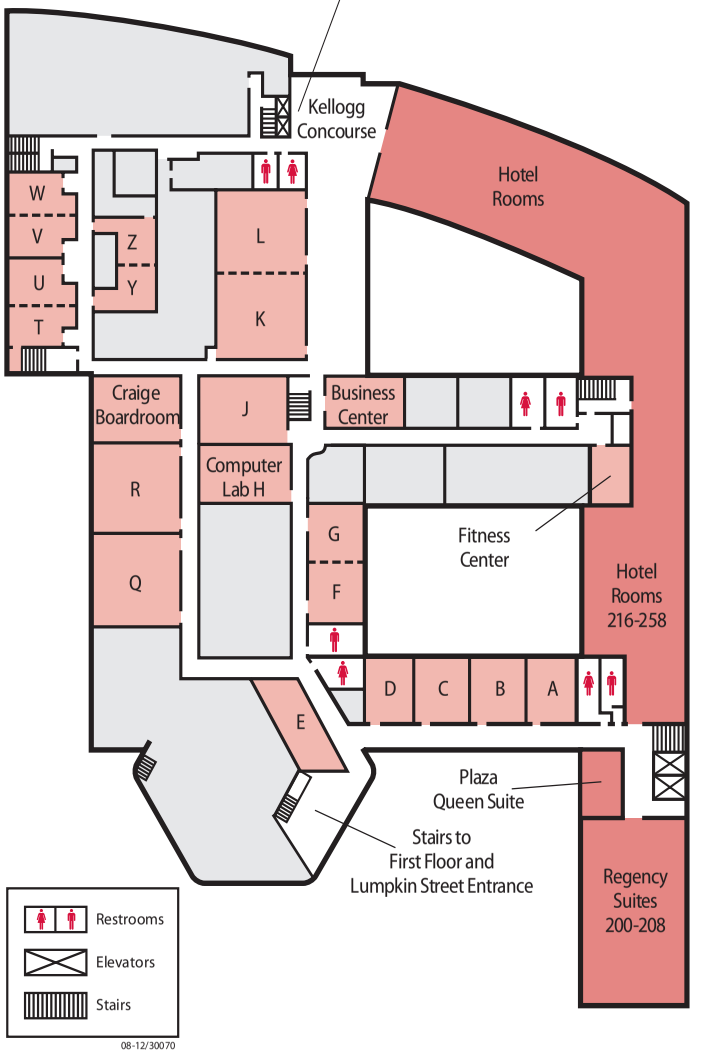

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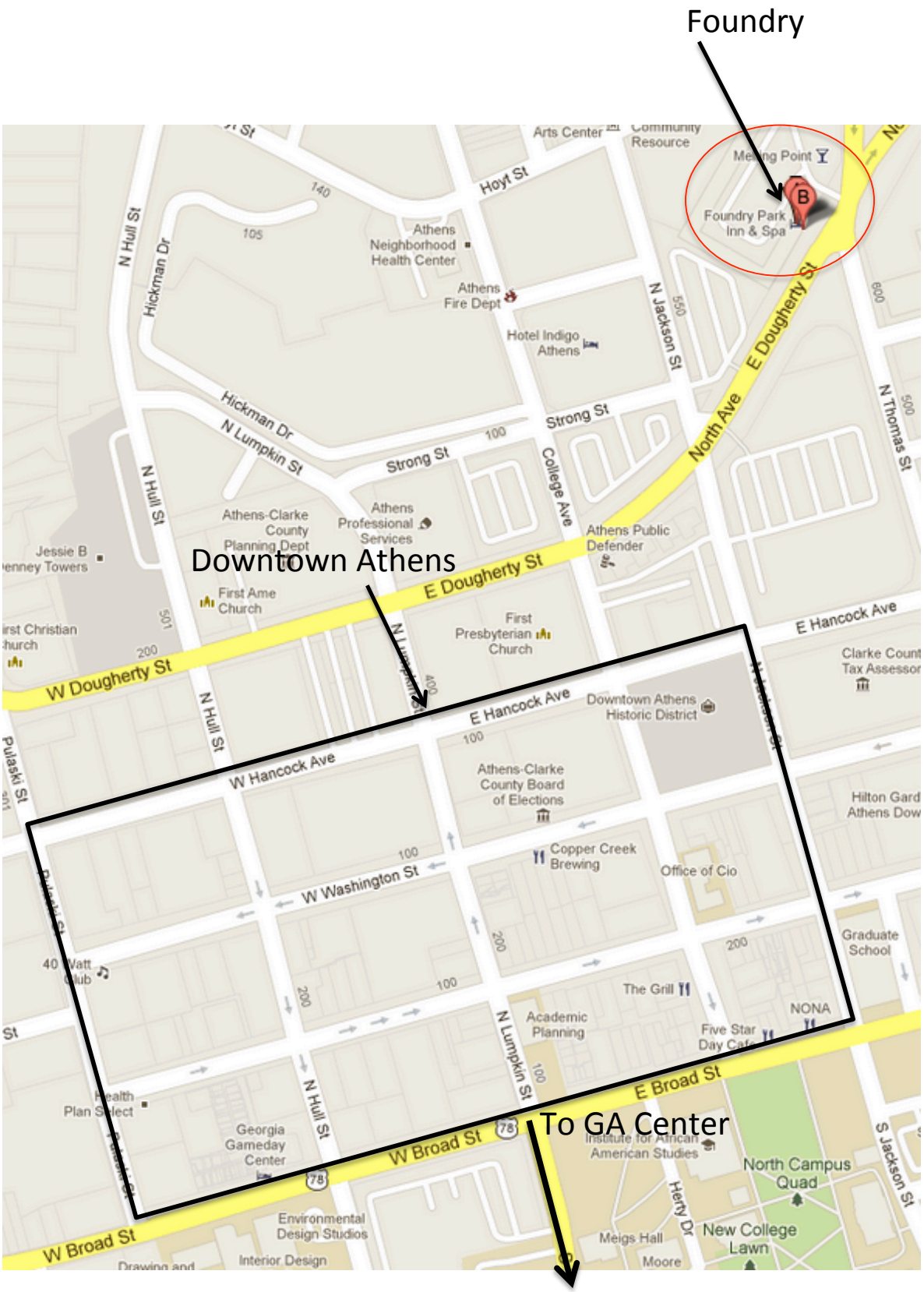

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If you have lodging at the UGA Hotel, you may unload your car at the front circle of the Hotel (Carlton Street), then park in the parking deck.

Assistance with your belongings will be provided by our Concierge attendants if needed. Your automobile may remain at this location while you register at the hotel desk but should be moved promptly to the parking deck after check in.

A daily flat-rate parking charge can be added to your room bill, if you wish, and you will be given a parking pay voucher to give to the parking attendant. This pay voucher will allow you in and out access for the duration of your visit.

Vans and other vehicles over seven feet tall cannot fit in the parking deck but arrangements can be made after arrival to secure outside parking.

Motorcycles are not permitted within the parking deck but may be parked near the main entrance to The UGA Hotel.

The South Campus Parking Deck is operated by the University of Georgia and is not governed by the Hotel.

The parking deck is open 24 hours.

Ground Transportation

Scheduled ground shuttle service and rental car services are available between Atlanta Hartsfield-Jackson International Airport and The UGA Hotel and Conference Center.

Shuttle Service to/from Atlanta Hartsfield-Jackson International Airport

- Athens Town Car: 706-255-9643
- Bulldog Limousine: 706-613-5206
- Groome Transportation: 706-612-1155
- Southeastern Stages: 706-549-2255

Athens Taxi Services

- Five Star Taxi: 706-549-2224
- Golden Taxi: 706-543-5646
- The Limousine Company: 706-227-1324
- Top Dawg Taxi Service: 706-552-0744
- United Taxi: 706-549-0808
- Your Cab: 706-546-5844

Athens Auto Rental Companies

- Avis: 706-543-3904
- Budget: 706-353-0600
- Enterprise: 706-546-8067
- Hertz: 706-543-5984
- U-Save Auto Rental: 706-548-4960

UGA Hotel Shuttle Service is free to hotel guests and serves the local Athens area, including the UGA Athens Campus and Ben Epps Airport. Call the Concierge Desk: 706-542-1932.

UGA HOTEL
and conference center
AT THE GEORGIA CENTER

UGAHOTEL.COM 800.884.1381

Notes

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Notes

Important Phone Numbers

Emergency

911

Athens-Clarke Police, http://www.accpd.org/

706.613.3330

Athens Convention and Visitors Bureau, http://www.visitathensga.com/

706.357.4430 | 800.653.0603

Classic Center convention center, http://www.classiccenter.com/

706.208.0900 | 800.918.6393

Directory Assistance

411

Hospital: Athens Regional Medical Center, http://www.athenshealth.org/

706.475.7000

Hospital: St. Mary's Health Care System, http://www.stmarysathens.org/

706.389.3000

Time and Temperature

706.548.2253

Tourist Information: Athens Welcome Center, http://www.athenswelcomecenter.com/

706.353.1820 | 866.455.1820

University of Georgia Directory Assistance, http://www.busfin.uga.edu/directory/

706.542.3000

University of Georgia Police, http://www.police.uga.edu/

706.542.2200

University of Georgia Sports Tickets, http://www.georgiadogs.com/

706.542.1231