KIRK WANGENSTEEN, MD, PhD LAB UPDATE

Kirk Wangensteen has been busy setting up and moving into his new office and laboratory space on the 9th floor of BRB. He has been promoted to Assistant Professor, tenure-track, with the support of his post-doctoral mentor Klaus Kaestner, and from Anil Rustgi and the GI division, and he has received a generous start-up package to kick-start his research program. The Wangensteen Lab aims to develop new understanding and treatments for liver diseases such as toxic liver injury and liver cancer. The lab employs mouse models to elucidate genetic mechanisms of liver regeneration and cancer. The work in the lab will use novel methodologies – which were developed by Drs. Wangensteen and Kaestner – to perform genetic screens in live mouse livers during regeneration and cancer initiation. The ultimate goal is to discover new drug targets for the treatment of liver diseases.

Kirk is accompanied by Noam Erez, M.Med.Sc., in the move from Dr. Kaestner’s lab. Noam is a research specialist who has been working with Kirk for the past 1.5 years, and who has about a decade of lab research experience. Kirk is also joined by post-doctoral fellow Julia Kieckhaefer, PhD, who recently completed her graduate studies in Dr. Kaestner lab. Julia’s PhD thesis project examined the role of several genes in intestinal biology. The lab is accepting additional post-docs and graduate students. Please welcome Kirk, Noam, and Julia to the floor, and learn more about the Wangensteen laboratory at their website: https://www.med.upenn.edu/wangensteenlab.

THE MOLECULAR PATHOLOGY & IMAGING CORE UPDATE

The Molecular Pathology & Imaging Core (MPIC) is excited to announce that they recently acquired a Leica BOND RXm auto-stainer, which will be used for automating IHC staining. The BOND RXm will work with both paraffin and frozen sections. The BOND RXm will deparaffinize the paraffin sections, perform antigen retrieval, and staining with DAB. When the run is finished the only thing that you’ll need to do is dehydrate the slides and add a coverslip.

The equipment has 3 trays that can hold 10 slides. Each of the 3 trays work independently of each other and, therefore, 3 different users can be using the equipment even if they start at different times. Researchers will be trained on the equipment, a user account will be created, and from there the user will be able to make use of the equipment whenever it’s available. The researcher will need to supply their primary antibody, but after that the rest of the reagents will be provided. The cost will be based on the number of slides stained and will cover the cost of reagents. For any additional information, please contact Adam Bedenbaugh, blakebe@pennmedicine.upenn.edu.
CONGRATULATIONS

Kenneth S. Zaret, PhD
Joseph Leidy Professor
Department of Cell and Developmental Biology
Perelman School of Medicine University of Pennsylvania

For winning the Stanley N. Cohen Biomedical Research Award, one of Penn Medicine’s Awards of Excellence!

“The distinguished awardees exemplify our profession’s highest values of scholarship and teaching, innovation, commitment to service, leadership, and dedication to patient care. They epitomize the preeminence and impact we all strive to achieve. The awardees range from those at the beginning of their highly promising careers to those whose distinguished work has spanned decades.”

GENETICALLY-MODIFIED MOUSE CORE UPDATE

Several years ago the University of Pennsylvania School of Medicine funded the creation of a dedicated mouse embryo cryopreservation storage facility. This facility is located in a secured room in the Anatomy-Chemistry Bldg and is overseen by the Core. The facility currently contains 9 liquid N\_2 storage tanks (plus a working tank in the microinjection room) with alarms and a source tank for liquid N\_2 refills. The Core bears responsibility for maintaining the integrity of the N\_2 dewars, and maintaining up-to-date, computerized storage records that can be accessed in real time by P.I.s. There is a modest per line yearly charge to Core users for this cryostorage service ($24/line/yr for center members). The fee will be collected quarterly through our database according to the account number that the user provides. Users can monitor their inventories and arrange with the Core to have samples sent to collaborators. The user needs only to provide the contact info for the recipient and the name of the line to be sent and the Core will take care of the shipping process.

The Transgenic and Chimeric Mouse Facility began providing direct genome editing services using the CRISPR-cas9 technology two years ago. CRISPRs (clustered regularly interspersed short palindromic repeats) encode RNAs (guide RNA) target a CRISPR-associated protein (Cas9) to cleave a DNA sequence in a site-specific manner. Following the double-stranded break, non-homologous end joining generates targeted deletions of random size. The DNA cleavage can also be used to enhance high-fidelity homologous recombination using a co-injected single stranded or double stranded DNA template. These CRISPR/Cas9-based methodologies significantly reduce the time and resources involved in generating genetically modified mouse lines.

The direct genome modification service based on CRISPR-Cas9 was integrated into the Core services in 2014 and has rapidly increased in its utilization. The overwhelming majority of the projects use an injection mix of Cas9 RNA and sgRNA either with or without template DNAs. The mix is injected into the cytoplasm of fertilized mouse oocytes of the strain of choice requested by the user. Similar to the DNA injection service, injected eggs are cultured O/N and the embryos are surgically transferred into pseudopregnant females and allowed to go to term. For ‘knock-in’ and targeted sequence modification projects, the eggs are cultured O/N in the presence of 50 uM SCR7, an inhibitor of Ligase IV to enhance homologous recombination (vs. non-homologous end-joining) events. The success rate for the KO projects ranges from 5%-50% of the live-born with frequent occurrence of bi-allelic mutations. KI projects based on homologous recombination remain less successful (3-10%) and the success frequency varies tremendously based on multiple variable in the project (base substitution, LoxP or tag insertion, large segment insertion). The Core continues to monitor the outcome of all projects and collect data that would help improve the efficiency of this technology.

Please remember to cite the Center (NIH-P30-DK050306) and its core facilities (Molecular Pathology and Imaging Core, Host-Microbial Analytic and Repository Core, Genetically-Modified Mouse Core, and Cell Culture and iPSC Core) in your publications.