

INSTITUTIONAL BIOSAFETY COMMITTEE MEETING

August 24, 2016

3 PM, 410 Plant Biotechnology Building

MEMBERS PRESENT: Chair, Jun Lin; Vice Chair, Patti Coan; David Bemis, Tamara Chavez-Lindell, Paul Dalhaimer, Doris D'Souza, Reza Hajimorad, Elizabeth Fozo, Brittany Isabell, Reggie Millwood, Deidra Mountain, Jae Park

Ex-Officio – Linda Hamilton, Scott Moser, Brian Ranger, Jessica Woofter

MEMBERS ABSENT: Seung Baek, Al Iannacone, Melissa Kennedy, Ling Zhao

OTHERS PRESENT: Dr. Bruce McKee, Dr. Thomas Denes

Opening:

The meeting was called to order by the Chair, Jun Lin at 3:00 PM. The minutes of August 3, 2016 were reviewed and approved as written (two abstentions).

IBC Applications:

#238-16 (Feng Chen) Infectious Agents & Recombinant DNA Registration, III-E-2-a, 3-year rewrite

Dr. Chen's research employs functional genomics and transgenic plant development to study various plant genes involved in resistance to insect pests or plant biomass production (bioenergy studies). Methyltransferase, acyltransferase, terpene synthase and other cell wall-related genes derived from plants (rice, poplar, tobacco, soybean and Arabidopsis), fungi (e.g. *Metarhizium spp.*), and amoebae (e.g. *Dictyostelium discoideum*) will be subcloned and assayed for specific enzymatic activities. Genes of interest will be cloned and mobilized onto binary vectors for Agrobacterium-mediated transformation of Arabidopsis, tobacco and soybean. GUS and GFP will be used as reporter genes. Transformants are to be further analyzed and regenerated. The registration also included the culturing of *Klebsiella pneumoniae* (environmental isolate; non-CRE strain) to be used as food for cultured amoebae. The committee approved the registration pending clarification of the source and specific strain characteristics of *K. pneumoniae*; storage of *K. pneumoniae* cultures; completion of BSL-2 training; and correction of minor typographical errors. Containment was set at BSL-1 for molecular biology/plant procedures and BSL-2 for *K. pneumoniae* culturing and associated procedures.

#350-16 (Paul Frymier) Recombinant DNA Registration, III-E, 3-year rewrite

Dr. Frymier's research focuses on optimizing light-induced hydrogen production in prokaryotic systems. Briefly, photosystem I from *Synechocystis sp.* PCC6803 and various hydrogenase genes from *Ralstonia eutropha* are being hybridized (by traditional molecular techniques) so that light-induced hydrogen production can happen at a faster rate. Ultimately, the hybridized complex will be expressed in *R. eutropha*. The containment level was established at BSL-1. The committee approved the registration pending revision of typographical errors and updates to training information.

#352-16 (Liz Fozo) Infectious Agents & Recombinant DNA Registration, III-D-1-a, 3-year rewrite

Dr. Fozo's research investigates the role that genes encoding small regulatory RNAs (sRNAs), small proteins, and membrane fatty acids play in the Risk Group 2 pathogens *Escherichia coli* O157:H7 and

Enterococcus faecalis; specifically, their role in survival/growth under extreme environmental conditions and in inducing disease. Briefly, mutants will be generated using standard recombinant DNA/molecular techniques (e.g. temperature-sensitive recombination systems; constructs to generate fatty acid gene deletions in *E. faecalis* delivered via conjugation) to disrupt the target genes with selectable marker genes. Similarly, fluorescent reporter genes (e.g. mCherry) will be used to replace the target gene so that gene expression can be monitored. Mutants will then be examined for any growth defects compared to the wild type organism. Genes of interest may also be overexpressed in *E. coli* MG1655 under native or inducible promoters. The containment level was established at BSL-2. The committee approved the registration as written and there was one abstention.

#442 (Heidi Goodrich-Blair) Infectious Agents & Recombinant DNA Registration, III-D-4-a, New Registration

Dr. Goodrich-Blair's research investigates the naturally occurring tripartite interaction between invertebrate nematodes in the genus *Steinernema*, their mutually beneficial bacterial symbionts in the genus *Xenorhabdus*, and the larval stage invertebrate insects these pairs of organisms infect. Using standard bacterial genetic, molecular, and biochemical techniques, bacterial genes and gene products that play a role in either the beneficial or the pathogenic host interactions are identified and characterized. Briefly, mutants and recombinant strains of *Xenorhabdus spp.* are generated by transposon or site-directed mutagenesis, and the desired genetic alteration is typically obtained using selectable markers (e.g. antibiotic resistance genes). Mutants are assayed for their ability to associate with the nematode host (mutualism), or to suppress immunity and kill an insect host. *Salmonella typhimurium* and *Enterococcus faecalis* are used for controls in various assays. The committee approved the registration pending clarification of the genetically modified organisms listed under rDNA-E-4 (pg. 3). Containment was set at BSL-1 for molecular techniques and procedures involving *Xenorhabdus spp./nematodes/invertebrate* insects. BSL-2 containment will be used for procedures involving *S. typhimurium* or *E. faecalis*.

#443 (Bruce McKee) Recombinant DNA Registration, III-D-4-a, New Registration

Dr. McKee's research aims to develop better genetic and cytological tools to analyze the proteins involved in meiotic chromosome segregation in *Drosophila melanogaster*. Specific aims include 1) tagging meiotic proteins so that they are targeted for auxin-induced proteasomal degradation; 2) optimizing delivery of detectable markers used for immunocytology and protein purification; and 3) introducing targeted mutations at such a high frequency that bi-allelic gene disruption can be achieved in a target tissue in nearly 100% of cells (e.g. essential genes with likely roles in chromosome segregation, such as cohesins, condensins, histone chaperones, etc.). Standard molecular tools for generating transgenic *Drosophila* will be used, including targeted gene editing via CRISPR/Cas9. The committee approved the registration as written. Containment was set at BSL-1.

#444 (Thomas Denes) Infectious Agents & Recombinant DNA Registration, III-D-1-a; 2-a, New Registration

Dr. Denes' research focuses on the effects of phage resistance, bacterial envelope composition, and changing environmental conditions on the fitness and physiology of bacterial foodborne pathogens such as *Salmonella spp.* (non-typhoidal) and *Listeria monocytogenes*. Briefly, gene deletions will be created by overlap extension PCR delivered via temperature-sensitive suicide vectors. Deletion phenotypes will be confirmed through complementation with the wild-type gene (standard molecular techniques). Target genes include those encoding membrane-associated proteins which may play a role in phage resistance and/or antibiotic resistance. Other techniques include subculturing, phage/bacteria enumeration, DNA/RNA extractions, and lectin binding assays. The committee approved the registration as written. Containment was set at BSL-2.

Old Business:

Administrative Report

i. Contingency updates:

Brian Ranger provided the administrative report. Following up on the August 3, 2016, IBC meeting, Dr. Elena Shpak's registration (#313-16) was updated to include a clarification that ERECTA, ERL1, ERL2, and TMM are the primary genes being assayed. Dr. Michael Karlstad's registration (#349-16) was administratively revised, including: removal of the *in vitro* study objectives from the Non-Technical Summary; clarification of pathogen strain characteristics; and the correction of biosafety cabinet certification dates. Dr. Steve Wilhelm's (IBC #404-16) biosafety cabinet will be recertified in November (research objectives requiring a BSC will not commence until after the recertification). Dr. Colleen Jonsson's registration amendment (#434) was corrected administratively (typos/syntax). Dr. Jeremiah Johnson's registration (#441) was corrected to include a list of control pathogens in the Non-Technical Summary; pathogenicity characteristics of the *L. monocytogenes* PrfA mutant; and the biosafety cabinet recertification date (8/5/16).

ii. Administrative Approvals:

The following 3-year renewals for human-derived materials were approved by the Biosafety Office:

- Dr. Reba Umberger (#405-16): cytokine testing (ELISA) on primary human plasma samples;
- Dr. Shanfeng Wang (#407-16): use of primary human fetal neural progenitor cells (Harvard Medical School) in various adherence, proliferation, and differentiation assays using a variety of engineered substrates;
- Dr. Jiangang Chen (#408-16): use of human kidney, breast, and prostate cell lines (ATCC) to test estrogenic and androgenic properties of environmental compounds.

iii. Administrative Terminations

Dr. J. Lannett Edward's registration (#351-13) was administratively terminated as her experiments have concluded. Recombinant constructs are to remain in secure storage at JARTU.

iv. Accidents, Injuries/Exposures:

There was one reported needle stick injury at the College of Veterinary Medicine. The technician punctured her finger with a needle used to collect a fine needle aspirate of dog lymph node. The injury occurred when trying to remove the capped needle (sample collection container exemption) from the luer lock. The cap released suddenly causing a rebound reflex injury. The puncture was flushed, and the technician sought medical care per exposure response protocol. The corrective actions will include the use of mechanical devices (e.g. forceps) for removing needle caps from collection syringes.

v. Laboratory Report

Linda Hamilton gave a brief update to the committee regarding recent laboratory audits. She is still working on the BSL-1 labs. Linda noted that a common finding in BSL-1 labs is unnecessary recapping of needles (some used in conjunction with biological material, some used for dispensing chemical solutions or other applications). Resultantly, general reminders for sharps safety should be issued. She also received a suggestion to have the Biosafety Office (and other safety offices) push out quarterly reminders relative to safety and compliance.

iMedRIS Update

Jessica Woofter notified the committee that she is preparing a draft iMedRIS manual for review at the next meeting.

Annual Report (FY16)

Brian notified the committee that the Annual Report is posted to the Biosafety website.

Charter Refresh

Brian notified the committee that he is still working on the charter refresh and will start working towards standard operating procedures for the committee.

New Business:

Other

Brian notified the committee about the Biosafety Office work-study student.

The meeting was adjourned at 3:57 PM. The next meeting is tentatively scheduled for September 21, 2016, 3 pm.