### The Emory Gnotobiotic Animal Core - FACILITIES AND RESOURCES

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**Fields Relevant for the Emory Gnotobiotic Animal Core (EGAC)**

**EMORY MICROBIOME CORE FACILITIES**

The **Emory Integrated Core Facilities** (EICF) support microbiome studies by synergizing the skills and resources of three core facilities, namely **The Emory Gnotobiotic Animal Core(EGAC)**, **The Emory Integrated Genomics Core** (EIGC), and **The Emory Integrated Computational Core(EICC)**. Workflows have been optimized between the three cores to form a pipeline whereby gnotobiotic studies are undertaken within the EGAC, sequencing of microbiome 16S rDNA from gnotobiotic studies undertaken by the EIGC, followed by expert analysis of the generated sequence data and characterization of the microbial alpha and beta diversities undertaken by the EICC.

**EMORY GNOTOBIOTIC ANIMAL CORE (EGAC)**

Introduction**:** The **Emory Gnotobiotic Animal Core (EGAC)**, one of the **Emory Integrated Core Facilities (EICF),** is located in the five-story, 200,000-square-foot Health Sciences Research Building (HSRB), situated in rooms next to the Specific Pathogen Free (MPF) murine housing, as well as the Transgenic Mouse and Gene Targeting Core. The facility contains sixteen 3’ foot wide rigid isolators (Parkbio), each with the capacity to house 12 mice cages each. Each cage has a maximum capacity of 5 mice per cage. Class II biological cabinets are available to investigators for experimental use.

Animal welfare: The facility has a dedicated technician to monitor murine health. The facility is run under Emory University’s IACUC approved Standard Operating Procedures which oversees and certifies that care and use of the animals is ethical and humane. The IACUC oversight is implemented by an ongoing review and approval of the facility by way of written animal use protocols submitted for review by IACUC committee members. In addition, a semiannual inspection of all areas where animals are housed or undergo procedures is undertaken within the facility. Federal regulations, veterinary standards of care, campus policies, and facility standard operating procedures are used by the IACUCs in their evaluations of animal use.

Microbiological Testing: We employ rigorous microbiological testing of all autoclaved material entering the isolators, including the food, water and bedding. We also regularly monitor germ-free mice within isolators

and periodic control necropsies. Our tests include **1)** real time quantitative PCR amplification of the V4 region 16S rRNA gene, **2)** plating of fecal samples on BBL Brain Heart infusion with 10% Sheep *Blood plates* (nonselective controls for all strains) and incubation at 37ºC for 7 days, **3)** Incubation in Thioglycollate medium with indicator, a medium used for the isolation and cultivation of aerobes, anaerobes and microaerophiles that are not excessively fastidious, and **4)** Gram stain, which is a general visual examination for the presence of bacteria, and distinguishes between Gram-positive and Gram-negative organisms.

Gnotobiotic colonization:For colonization studies, 5-week-old germ-free mice are be inoculated with a either **1)** a single microbe (mono-colonization) with a single bacterium), or **2)** a defined finite group of microbes such as **altered Schaedler flora** (ASF) is a community of eight bacterial species: two Lactobacilli, one Bacteroides, one spiral bacteria of the Flexistipes genus, and four extremely oxygen sensitive (EOS) Fusobacterium species. The bacteria are selected for their dominance and persistence in the normal microflora of mice, and for their ability to be isolated and grown in laboratory settings. Germ-free animals, mainly mice, are infected with ASF for the purpose of studying the gastrointestinal (GI) tract. The standardized microbial cocktail enabled the controlled study of microbe and host interactions, role of microbes, pathogen effects, and intestinal immunity and disease association. Also, compared to germfree animals, ASF mice have fully developed immune system, resistance to opportunistic pathogens, and normal GI function and health, and are a great representation of normal mice, or **3)** a polymicrobial population sourced from another mouse or a human clinical sample, for example a humans or animals that has been treated with an antibiotic which may be used to establish whether it is the altered microflora diversity that triggers changes in organismal physiology. Microbes may be introduced directly into the stomach with a 24-gauge ball-tipped gavage needle, or because mice are coprophagic, polymicrobial microbiome transfer from mouse to mouse may be done by introducing fecal pellets into the recipient cage. Routine microbiological testing to establish that the desired microbiome diversity is maintained throughout the experiment is done collecting stool samples, purification of DNA from the samples, and PCR amplification of the V4 region of the 16S rRNA gene from the sample. The PCR product are then sequenced and output files processed using the QIIME and MOTHUR pipelines. In addition, to gnotobiotic isolation within isolators, the facility will be equipped with the Tecniplast ISOcage system allowing gnotobiotic isolation at 36 single cage level, enabling multiple studies on the same rack.

ISOcage Tecniplast Bioexclusion System:Precise modulation of the microbiome is increasingly been appreciated as feasible approach to positively impact health and disease. However, heterogeneity of the microbiome between murine colonies housed in various facilities nationwide represent a tangible challenge to scientific rigor and reproducibility. Indeed, incongruent results have been observed between facilities when mice of the same phenotype were subjected to the same assays. We have developed a facility to directly control for microbiome heterogeneity by, where feasible, undertaking our assays in mice gnotobiotically colonized them with a defined flora. We undertake this by housing our mice inwithin our cutting edge Tecniplast ISOcageP Bioexclusion system. These are airtight individual mouse cages with high positive pressure that are specifically designed for germ-free, gnotobiotic and bioexclusion studies. The ISO cage system is the latest design for gontobiotics and germ-free animals because it allows researchers to undertake up to 36 simultaneous gnotobiotic studies, compared to only one study at a time in conventional multi-cage gnotobiotic isolators. For gnotobiotic experiments, mice can be initially raised under germ-free conditions. After weaning (3 weeks) mice will be transferred to an ISOcage cage.

For experiments with a fully characterized controlled microbiome background, mice may be gnotobiotically colonized with Altered Schaedler Flora (ASF). ASF is a standardized microbial blend of eight bacteria, which allows for the exquisitely meticulous studies in a fully defined microbiome background. Importantly, compared to germ-free animals, ASF-associated mice have normally developed immune system, they have resistance to opportunistic pathogens, and normal gastrointestinal health. They are thus an excellent representative model of normal mice with a defined microbiome. ASF colonization is done by the initial purchase of fecal pellets containing ASF from Taconic Biosciences Inc., which is a commercial supplier of gnotobiotic animals and materials. For probiotics supplementation experiments, ASF colonized mice are fed twice a week and housed in the Tecniplast ISOcageP Bioexclusion system. By this method we can achieve complete scientific reproducibility by undertaking our experiments with a defined background microbiome.  For experiments in this proposal, we will undertake fecal microbiome transplant experiments by isolating the samples from the luminal content od humans. It is imperative that following transplantation, the mice be maintained in bioexclusion conditions using the Tecniplast ISOcageP Bioexclusion system for the duration of the experiment (up to 8-weeks). This approach will eliminate the possibility of any further microbial components entering the transplanted microbiome.

Murine strains bred and held within the facility:The facility currently has two large breeding colonies to maintain strains of C57BL/6J.

Transferring new strains into the facility:Currently, we accept rederived germ-free mice from approved vendors, such as (but not limited to) Taconic Biosciences and The National Gnotobiotic Animal Resource Center at UNC. Mice must be shipped in approved transporters. Mice are transferred into newly set up isolators, and kept as the only genotype in said isolator for 4 weeks, and monitored weekly for germ-free status.

Dedicated gnotobiotic barrier access suite*:* For this proposal, we will undertake bone fracture studies in germ-free and gnotobiotic conditions. GF and gnotobiotic mice used in manipulations will be housed within our cutting edge Tecniplast ISOcageP Bioexclusion system**.** GF and gnotobiotic mice will be transferred within the hermetically sealed ISO cage to a dedicated axenic *Biological* Safety *Cabinet*where fracture procedures will be undertaken.