

## POTENTIAL PROJECTS INVOLVING LABORATORY OPOSSUMS FOR UTRGV UNDERGRADUATE STUDENTS

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**BACKGROUND:** The UTRGV laboratory opossum breeding colony consists of a steady state of approximately 1,400 gray short-tailed opossums (*Mondelphis domestica*). Several thousand opossums are produced annually for perpetuating genetic stocks and inbred strains and conducting research. One of the unique advantages of laboratory opossums is that they are born at the developmental stage of a human embryo at 6 weeks of gestation, allowing easy experimental manipulation of embryos and fetuses.

Research programs in progress at UTRGV include genetics of diet-induced hypercholesterolemia and consequent atherosclerosis, genetics of diet-induced non-alcoholic fatty liver disease (steatohepatitis), development of the nervous system including neuropathologies and behavioral disorders, and the pathological consequences of infection by Zika virus (including intra-cranial inoculation of embryos and fetuses with Zika virus).

Management of the breeding colony and research conducted with animals from it provide opportunities for student research projects which might be conducted for credit within a course or as a non-credit activity aimed at enhancing skills and capabilities. It is envisioned that each project will provide opportunities to gain experience in experimental design, literature searching, hands-on research, data analysis, abstract and poster preparation, and oral presentation of results (mini-seminar). Some projects would expose students to the preparation of IACUC protocols, and some might lead to authorship on publications.

Some examples of questions that potential research projects could be designed to answer are as follows.

### RESEARCH RELATED TO HUSBANDRY (DIRECT CONTACT WITH ANIMALS):

1. What are the levels of ammonia and humidity in the ventilated cages of the typed used at UTRGV during the days and weeks after changing, and how often should cages be changed to maintain those levels below acceptable limits? Variables that could be examined are:
  - a. Number of animals of various age classes in a cage
  - b. Type and amount of bedding
  - c. Size of cage
2. At what ages do behavioral changes that lead to aggression occur to the extent that juvenile females and males should be separated from one another, and at what age does within-sex aggression mandate that juveniles be placed in individual cages? Variables that could be examined are:
  - a. Number of animals in a cage
  - b. Size of cage
  - c. Strain of opossum
3. Under the standard diet fed ad libitum, what factors affect growth rates of juvenile and young adult males and females? Variables that could be examined are:
  - a. Size of cage
  - b. Animal density within cages
  - c. Presence or absence of opposite sex in the cage
  - d. For young adults, opportunity to mate
  - e. For young adults, exposure to pheromones (continuously or periodically) through a cage divider, but without opportunity to mate
4. (Related to item 3) Is there a postnatal growth developmental checkpoint between weaning and sexual maturity? Variables that could be examined are:
  - a. Exposure to the opposite sex
  - b. Litter size
  - c. Strain of opossum
5. Does fasting nursing mothers in preparation for anesthesia and experimental procedures adversely impact litter survival? Variables that could be examined are:
  - a. Age of litters
  - b. Size of litters
  - c. Duration of fast (e.g., 6 or 16 hours)

6. Do older animals show evidence of osteoarthritis in histological preparations of knee cartilage? Variables that can be examined are:
  - a. Strain
  - b. Body weight
  - c. Bone density

#### RESEARCH INVOLVING ANALYTICAL PROCEDURES IN THE LABORATORY (NO DIRECT CONTACT WITH ANIMALS)

1. DNA methylation (for an exceptional student who is highly enthusiastic about lab research)

DNA methylation is an epigenetic mechanism used by cells to control gene expression. Despite being widely studied among eutherian (“placental”) mammals, no study has ever examined the methylation status of entire marsupial genomes. Only three studies have examined the methylation status of chromosomes (see below) and mostly in the context of X chromosome inactivation. In this project, the student would use next generation sequencing data collected for 576 animals to study methylation patterns in the genome of the laboratory opossum. In particular, the student would retrieve positional information about certain restriction enzyme sites present in its genome and compare it with a database of sites that are present in our dataset. The sites that are not present in our dataset can be assumed to be methylated in all 576 animals, since we used methylation-sensitive restriction enzymes.

##### References

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O’Neill RJ, O’Neill MJ, Graves JA. Undermethylation associated with retroelement activation and chromosome remodelling in an interspecific mammalian hybrid. *Nature*. 1998;393:68–72.

Loebel DA, Johnston PG. Analysis of DNase 1 sensitivity and methylation of active and inactive X chromosomes of kangaroos (*Macropus robustus*) by in situ nick translation. *Chromosoma*. 1993;102:81–7.

2. Sex determination based on next generation sequence data

Next generation sequencing data opens up the possibility of assigning the sex of an individual for whom sex isn’t known (or was lost). The student will help develop (and test) different approaches of sex determination based on chromosomal heterozygosity measurements. Questions that can be asked are:

  - a. How accurate are the different methods of sex determination?
  - b. Which method is more reliable?