1. Mentor: Dr. Jamie Foster

2. Name of organization
Department of Microbiology and Cell Science, University of Florida

3. Title of the project
Microgravity-induced differential gene regulation in the symbiotic bacterium Vibrio fischeri

4. Abstract
The overall goal of this project is to examine the impact of microgravity on the cellular interactions between animals and beneficial microbes. Space flight causes numerous changes in the growth, physiology and virulence of animal-associated microbes. However, most studies have focused on pathogenic organisms, which comprise less than 5% of all microbes known to associate with animal tissues. The effects of microgravity on mutualistic microbes are virtually unknown. Here, in this summer project the student will help to examine the impact of space flight on gene expression in Vibrio fischeri, a beneficial microbe known to be essential for the normal development of the squid Euprymna scolopes. Specifically, the student will have two objectives:

A) Analyze previously generated bacterial transcriptome libraries. The intern will help to analyze four previously generated V. fischeri transcriptome data sets (i.e., all those genes expressed at a given time point) using bioinformatic and statistical analyses. The gene data sets were generated in the presence and absence of simulated microgravity using high-aspect-ratio rotating wall vessel (HARV) bioreactors. Two V. fischeri strains were used during the microgravity exposures including the parent strain V. fischeri and a mutant defective in the gene encoding Hfq, a critical regulator of bacterial virulence in the space environment. First, the recovered sequences will be screened for quality using the program Prinseq. The high quality sequences will be aligned against the publicly available V. fischeri genome, which will serve as a scaffold for recovered bacterial transcripts, thus providing information on the functions of the differentially expressed genes. All putative protein-encoding sequences will be compared with the NCBI non-redundant reference sequence database (RefSeq) using BLASTX. Those sequences identified with putative functions will be compared to the COG (Clusters of Orthologous Groups) databases for additional verification of function using the High Performance Computing cluster currently available at UF. The total number of reads for each putative protein will be tracked through all stages of analysis. Statistically different expression levels (i.e., relative abundances) will be determined using pair-wise comparisons between microgravity treatments and time points using the freeware program R.

B) Independently confirm the expression several key target genes in the transcriptome data set. Using the transcriptomic data, the student will help to confirm gene expression with quantitative real time polymerase chain reaction (RT-PCR). Cultures of V. fischeri will be incubated in the HARV chambers and sampled regularly (e.g. every 4 h) for 96 h. Samples will be preserved in RNA later and stored at -80°C until processing. Total RNA will be extracted from bacteria and converted to cDNA using commercially available kits. Primers will be designed using the Primer Express software...
(Applied Biosciences) and qPCR will be performed using a LightCycler 480 with a SYBR Green I Master kit (Roche). Based on the analysis of the data set and available time approximately five genes will be examined with qPCR analysis by the intern. Genes associated with quorum sensing (e.g. lux genes, autoinducer) and virulence (e.g. lipopolysaccharides, peptidoglycan) will be likely targeted.

5. **Expected contribution that the student will do on this project**
The intern would initially start by shadowing the mentor to learn the molecular biology procedures required to complete this project. Depending on the student’s experience level the student would be personally shown how to complete the task and then gradually left to continue the task independently. It will be expected that with the mentor’s guidance and training by the end of the internship the student will be trained in the described methods and computer programs.

6. **Expected student working hours (8 hours a day, 5 days a week)**
It will be expected that the student will work for a full 40-h workweek. However, depending on the specific procedures some days may be longer and others shorter. For example RNA isolation and clean up may require a 10 h day (with lunch), however, the student would be allowed to leave earlier on another day.