Abstract of proposed student project.

Background and Significance. Cardiac myosin binding protein C (MYBPC) is crucial in organization of the sarcomere and overall cardiac function. Targeted knockout of this gene in mice results in severe cardiac hypertrophy, disruption of myofibril alignment, and diminished cardiac function[1, 2]. Two separate mutations in the MYBPC gene have been identified in Maine Coon (A31P mutation)[3] and Ragdoll (R820W mutation)[4] cats, two breeds overrepresented for the development of Hypertrophic Cardiomyopathy (HCM). The A31P and R820W mutations identified in the MYBPC gene are specific to these two feline breeds and are hypothesized to have arisen separately as these two breeds are not thought to be related.

The population of cells in an anagen hair follicle such as a whisker contains many proliferating and terminally differentiated cells that express a variety of myosin and actin isoforms. These isoforms play key roles in mammalian whisker movement and response functions[5]. Collection of hair follicles of vibrissae is rapid and noninvasive. The risk of inadvertently growing a mixed cell population that includes epithelial cells is low, as the hair follicle resides within the dermal layer of skin. The successful isolation and differentiation of myogenic precursors from vibrissae would yield an *in vitro* model that can serve as an expandable, relatively low-cost platform, both for modeling genetic disorders and enabling further experimentation to develop therapeutics for feline HCM.

Specific Aim 1: Determine the efficacy of CRISPR sgRNAs designed to edit the feline A31P mutation. *Hypothesis.* CRISPR/saCAS9 will edit the A31P mutation with efficiency greater than 50%. *Approach:* Myogenic precursor cells from feline whiskers will be isolated and cultured. Primary cells will be transfected with sgRNAs into the cells using nucleofection, and efficiency of gene editing assessed using next-generation sequencing of amplicons. This is common practice for assessing efficacy of gene edits and our pipeline is in place with GenWiz to conduct such analysis.

Experimental methods and design. *Vibrissal bulb processing for cell isolation* - Hair bulbs obtained from whisker collections will be immediately processed using aseptic technique. Whiskers are washed three times with Hanks' Balanced Salt solution (HBSS). Using a micro-dissecting kit under a dissecting microscope each hair bulb is cut to separate the hair bulb from the hair shaft and flushed with cell dissociation solution (TrypLE) using a fine needle (27G x 3.4") in a 1 mL syringe. The solution containing the flushed hair bulbs is filtered and the cell suspension centrifuged. The cellular pellet is then separated from the supernatant and re-suspended in Skeletal Muscle Growth Medium (PromoCell) + 20% FBS + 1% GlutaMax supplement (ThermoFisher). The cells are then plated in a tissue culture dish coated with 10% Matrigel, and incubated at 37°C. The cells are checked every other day and the medium is changed twice per week. Our preliminary data suggest that the myogenic precursor cells are readily expandable. sgRNAs will be designed using SnapGene and software for CRISPR design. Synthetic probes will be ordered and transfected along with the saCAS9 enzyme into isolated myogenic precursor cells. We will evaluate the targeting efficiency first using Sanger sequencing and ICE/TIDE analysis and will use NGS to measure targeting efficiency more accurately. Our team has developed an effective screening strategy using RNP complexes with synthetic sgRNA introduced by nucleofection into cells and have identified considerable editing efficiency for some candidate sgRNA in canines.

The significance for Veterinary Medicine and the student. CRISPR/Cas9 gene editing has emerged as a powerful approach to understanding the roles of genes in all animals and humans. The FVSP student will learn how to culture cells from cat whiskers to obtain a primary cell culture for cell transfections. We have developed a small project which has a testable hypothesis and one that will move our larger project forward. The veterinary student will interact significantly with our team and will be trained by post-doctoral fellow Dr. Noble Noble is gene editing approaches which is important for advancing small animal medicine. Conducting both gene expression analysis, sequencing and transfection is feasible for a 3-month internship and Dr. Martyniuk and his team will work closely with the student to ensure success. We encourage trainees to present their research at the UF Genetics Institute symposium in the Fall as well as college wide events. Students will develop their own posters and present to the wider UF community, representing our college in research on campus which is very important.