

SUNY College at Oneonta

Animal Care and Use Protocol

Please complete all of the appropriate sections of this form and send an electronic copy to IACUC@oneonta.edu. Note that within one year of approval of the protocol the principle investigator must notify IACUC of either the completion the project or request an extension of the project.

Part A. Project Identification and Signatures

1. Type of Application: New protocol Renewal of # _____.
2. Project Title: iGEM SUNY Oneonta
3. Principle Investigator (PI):
 - a. Name (Last, First, MI): Fielhaber, Jill
 - b. Phone number: 607-436-3723
 - c. Campus address: 322 Perna Science
 - d. Email: jill.fielhaber@oneonta.edu
4. Person preparing this document:
 - a. Name: Sanchez, Helen
 - b. Phone: 832-855-9366
 - c. Email: sanchm41@oneonta.edu

Part B. Name of funding source/agency:

1. Grant number(s): Student Research Grants

Part C. Personnel – List the personnel who will be working on this study.

Name (Last, First, MI)	Phone number	Role in Project	Works with Animals	Years of experience
Sanchez, Helen		Wet lab		0
Hammes, Maddy		Wet lab		0
Curtin, Claire		Wet lab		0
Buchanan, Liam		Wet lab		0
Cordice-Little, Britney		Wet lab		0
Tarlen, Chloe		Wet lab		0
Ibraim, Asana		Wet lab		0
Rosa, Ashley		Wet lab		0
Walis, Sean		Wet lab		0
Kelly Gallagher	3180	PI		1
Jill Fielhaber	3723	PI	yes	6

Part C. Abstract (200 words). Provide an abstract describing the proposed work. Please use language that the layperson can understand. Emphasize the care and use of animals rather than detailed scientific methodology.

Beta Casein is the main proteins present in mammalian milk. Dairy cows can possess one of two alleles of the Beta Casein gene (CSN2). These alleles, termed A1 and A2, differ by a single nucleotide polymorphism, which results in a single amino acid substitution (Histidine – A1, Proline – A2). Recent

works have suggested that A2 milk is more tolerated by individuals with milk sensitivities, making the production of A2 milk an attractive target for small organic dairy farms, who must breed A2 cows naturally to maintain their organic status. The SUNY Oneonta iGEM team aims to develop a field deployable, rapid detection system to test for the A1 and A2 allele in cows. As part of the build, the team will need to develop methodologies for extracting DNA. To do this, we wish to obtain hair and saliva samples from Dairy cows located at a local organic dairy farm, with the permission of, and under the supervision of the owner of the cows. Once obtained, we will use these samples to test field DNA extraction methods and to validate our detector system.

Part D. Animal Request, Pain Class, Source of Animals (over three-year protocol period).

Common name	Pain class ¹	Number purchased Or received from other source	Number to be transferred from another protocol	Number produced in house	Total number
Cow	A	Max. 10 cows			10

¹Pain classes:

Class A: No pain or distress, or use of pain-relieving drugs (E.g., routine procedures requiring only transitory discomfort such as venipuncture, injections, ear tagging, or euthanasia, including anesthesia followed immediately by perfusion/exsanguinations/organ harvest resulting in death, etc.).

Class B: Potential pain/distress WITH appropriate analgesia/anesthesia/tranquilizers (E.g., any use of anesthesia/tranquilizers to restrain animals for procedures, surgery (survival or nonsurvival), tissue/organ collection before euthanasia, etc. Please complete Appendix A.

Class C: Pain/distress WITHOUT analgesia/anesthesia/tranquilizers. Procedures involving accompanying pain or distress to the animals and for which the use of appropriate anesthetic, analgesics or tranquilizing drugs would adversely affect the procedures, results or interpretation of data. Please complete Appendix A.

Part E. Housing.

1. Where will the animals be housed? N/A
 - a. Building and room number:

2. Will live animals be removed from the primary facility for any amount of time? N/A

Procedure(s) to be performed:	
Room and building:	
From where to where will animals be transported?	
Via what route will animals be transported?	
Who will transport animals?	
What equipment will be used to transport animals?	
At what time(s) of day will animals be transported	

Part F. Specific Aims and Details of Animal Use.

1. Background and significance (1-2 sentences):

Our project is focused on building a detection mechanism to test for A1 and A2 alleles in cows. This mechanism is centered around detecting the Single Nucleotide Polymorphism in the Beta-casein gene in cows.

2. Question addressed by the research (1-2 sentences):

Can we develop a field deployable genetic test that can be

3. How will the results of the research be used (1-2 sentences)?

If the project is successful, we hope to further develop the detector system to be deployed as a field genetic test for organic dairy farmers interested in producing A2 milk. This project will also be presented at the iGEM international competition, the 2020 Giant Jamboree.

4. Summarize the specific aims of the research (derived from the grant application if applicable):

We aim to develop a mechanism to detect the Single Nucleotide Polymorphism present in the beta-casein gene in cows. The SUNY Oneonta iGEM team will use the samples to:

1. test DNA extraction methods that can be readily used in the field
2. test the function of our detector system for determining the presence of A1 and A2 alleles in the animals.

5. Please provide a complete, sequential and accurate description of what procedures will be performed on/with the animals. You may include a diagram or chart to explain complex experimental designs and/or procedures. Please include the following information in your description. Use additional pages if necessary.
- a. For each species and treatment group within a study, describe all procedures performed on or with animals and indicate how often and when these procedures will be performed during the study. Identify pain classification (A,B,C) of each procedure as indicated in part C.
 - b. Stipulate how long (endpoint) animals will participate in the study; that is, end of experiment, sacrifice, etc. Stipulate dosages (mg/kg), routes and frequency of administration of any drugs used in the study.
 - c. Describe methods to be used in behavioral studies (including use of noxious stimuli or other methods of positive or negative reinforcement).
 - d. Briefly describe surgical procedures. Detailed descriptions of surgeries should be provided.
 - e. List for each species the experimental and control groups. Indicate number of animals in each and to which pain classification they are assigned.

Samples of hair and saliva will be obtained from a total of 10 cows. The cows will be selected randomly at the farm. We will start by obtaining the hair samples assuring that we obtain hair follicles. A total of 40-50 hairs will be plucked from different parts of the cow (hind leg and tail region) to ensure the least amount of discomfort.

Buccal DNA samples will be attained from each cow swabbing the inner cheek of the cow using a buccal or sterile cotton swab.

The samples will be obtained under the supervision from the cow owners to ensure safety of the animal and the personnel.

[https://www.beefresearch.ca/blog/cattle-tail-hair-dna-samples/#:~:text=20%20hairs%20will%20provide%20enough,breed%20associations%20require%2060%20hairs\)](https://www.beefresearch.ca/blog/cattle-tail-hair-dna-samples/#:~:text=20%20hairs%20will%20provide%20enough,breed%20associations%20require%2060%20hairs)

<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6060727/>

Part G. Justification of Species and Numbers.

Note: If the protocol involves more than one species, please complete Part E for each species used in the study.

1. Justification

a. Check all statements that apply to this protocol.

This model has been previously used.

- 1. Provide citation(s) and briefly explain why the citation is applicable to this protocol.**

This is a new model.

- 1. Describe the features of the species that make it desirable for this model and contrast it with other available models.**

The use of dairy cows is the focus of our study, since we are trying to identify the allele that produces A2 vs the A1 milk (most commercially available). Other species will defeat the purpose of our study.

2. How did you determine the number of animals required for the activities describe in this protocol?

a. Check all statements that apply to this protocol.

Pilot study. Group variances are unknown at present time.

Group sizes determined by statistical methods. Describe the method.

This is a pilot study and will be used to test field DNA extraction methods. We feel that 10 animals is sufficient as this will yield enough DNA samples to allow us to test different extraction procedures, but not so many as to become difficult to manage.

Group sizes are based on quantity of harvested cells or amount of tissue required. Explain how much tissue is required based on number of experiments and how much tissue you expect to obtain from each animal.

- Product testing. If the required number of animals is based on FDA guidelines, provide citation from the regulations, the IND tracking number or relevant FDA correspondence.**

- Other. Elaborate including method to determine group size.**

Part H. Monitoring Animal Health and Welfare.

- 1. List the study-induced or related adverse health conditions that animals might experience (i.e., pain, distress, health complications, etc.) as a result of their genotype or phenotype (Momentary pain or distress need not be described).**
 - a. How will pain and/or distress be monitored? List specific clinical signs as well as frequency of monitoring (including provisions for weekends and holidays).**

Pain and/or distress will be monitored by assessing any change in normal behavior of the cow. The owner of the cow or a designated responsible individual will be present during the collection of samples to monitor the well-being of the cow.

- b. Explain what steps will be taken to alleviate any pain, distress or discomfort that the animals might experience. Provide dose (mg/kg), route of administration, frequency and type of analgesic or tranquilizers to be administered. Also include any environmental changes (warming pads, soft bedding, fluids, etc.) that might be used in this regard.**

It is not anticipated that the animal will suffer any pain, distress or discomfort. If they do, the collection procedures will be transient.

Part I. Euthanasia/Disposition of Animals.

- 1. Identify specific endpoint(s) for each animal or group of animals used in this protocol. The endpoint is the point at which an animal or group of animals will no longer be used.**

Each cow will only be swabbed once to obtain saliva samples and a maximum of 50 hairs will be taken. Once this is done, the cow will no longer be used to collect any more samples.

- 2. Will the animals be euthanized at the end of the study?**

- No. Describe what will happen to the animals that are not euthanized upon completion of the activities described in this protocol.**

Upon completion of sample collection, the cows will return to their normal routine in their normal environment.

- 3. Will death or moribund (near death) condition be used as the experimental endpoint?**

- No**

Provide explanation and justification if answer is "Yes."

Part J. CITI training.

Personnel involved with animal research are required to complete one or more training courses that are pertinent to their work. Please indicate which courses you have completed.

Working with IACUC: Yes ___ No ___ Date: _____

Reducing Pain and Distress in Laboratory Mice and Rats: Yes ___ No ___ Date: _____

Working with Amphibians in Research: Yes ___ No ___ Date: _____

Working with Mice in Research: Yes ___ No ___ Date: _____

Working with Rats in Research: Yes ___ No ___ Date: _____

Working with Fish in Research: Yes ___ No ___ Date: _____

Other courses: (please list and indicate dates):

All personnel involved in the study have completed the Responsible conduct in research training provided through CITI within the last 2 years.

J. Fielhaber has completed "Working with IACUC" 12/31/2019

Part K. Signatures

Principal Investigator (PI): I hereby certify:

- The information provided in this protocol is accurate.
- No other procedures will be used in this project.
- Any modifications will be submitted for approval by IACUC prior to use.

Signature of PI

Date

Faculty Supervisor (If PI is a student): I hereby certify:

- This project is under my direct supervision.
- I am responsible for insuring that all provisions of protocol are complied with.

Name of Faculty Supervisor:

Campus Address and Telephone Number:

Signature of Faculty Supervisor

Date

Department Chair/Supervisor: My signature hereby indicates that I am aware of this proposal.

Name of Department Chair/Supervisor:

Campus Address and Telephone Number:

Signature of Department Chair/Supervisor

Date