Restoring Ecosystems and Biodiversity through Development of Safe and Effective Gene Drive Technologies
Monthly Technical Report
[Safe Genes Program]

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Project PoP: [5/1/2017-4/30/2021
Reporting Period: [12/11/17-01/12/18]

Briefing Prepared for Renee Wegrzyn

Project Overview

Problem: Invasive rodents cause biodiversity loss worldwide with impacts being particularly pronounced on islands. Rodents are also disease vectors and threaten food security through pre- and post-harvest losses.

Goal: Develop safe, controllable, and effective gene drive technologies in mice for potential application in eradicating invasive mouse populations on islands. As mice are the major mammalian genetic model, this research will also advance gene-drive approaches in rodent and other mammals more generally.

Key Aims:

- Develop and test first sex-biasing gene drive mechanisms in mammals including an innovative trans-effector drive
- Identify population specific, locally-fixed genetic targets for gene drive integration to develop and test spatial limitation of gene drive function
- Mathematically model gene drive function to inform development and testing in small populations in simulated natural environments
- Conduct hazard analysis and probabilistic ecological risk assessment of gene drives
- Conduct regulatory, stakeholder, and community engagement focused on potential gene drive application for biodiversity conservation

Accomplishments and Challenges to Date

Accomplishments (cumulative):

- Regulatory - FDA will be initial US regulatory agency with authority over this project
- Modeling
- Paper published in *Molecular Therapy* demonstrating efficient Y-chromosome shredding in ES cells
- "Target" founder mice generated & CMV-Cas9 imported and validated
- "gRNA (Cas9 version)" founder mouse generated and validated
- First zygotic homing experiments performed resulting in mosaic mice
- Vasa-Cas9, Vasa-Cpf1 and CMV-Cas9 transgenic mice generated

Challenges: ACURO approval process period

Technical Progress - Executive Overview

Technical progress update:

- Engineering of t-Sry mice (3.1.1.1): The induced pluripotent stem (iPS) cells were frozen over Christmas break. There are now several vials as back up in the freezer. Primary embryonic stem (ES) cells are also now being generated from a cross between a 129 female and a tw2/+ male.
- Generation 1 CRISPR Drive (3.1.1.2): Completed initial analysis of Generation 1 CAS9 zygotic homing strategy.
- Modeling (3.1.3): Refinement and write-up of modeling of locally fixed allele impacts on gene drive function on island and mainland populations.
- Regulatory Engagement (3.1.4): No significant progress to report
- Stakeholder Engagement (3.1.5): Stakeholder Interview Protocol finalized and Stakeholders prioritized

Milestones and Task Status Overview

Restoring Ecosystems and Biodiversity through Development of Safe and Effective Gene Drives

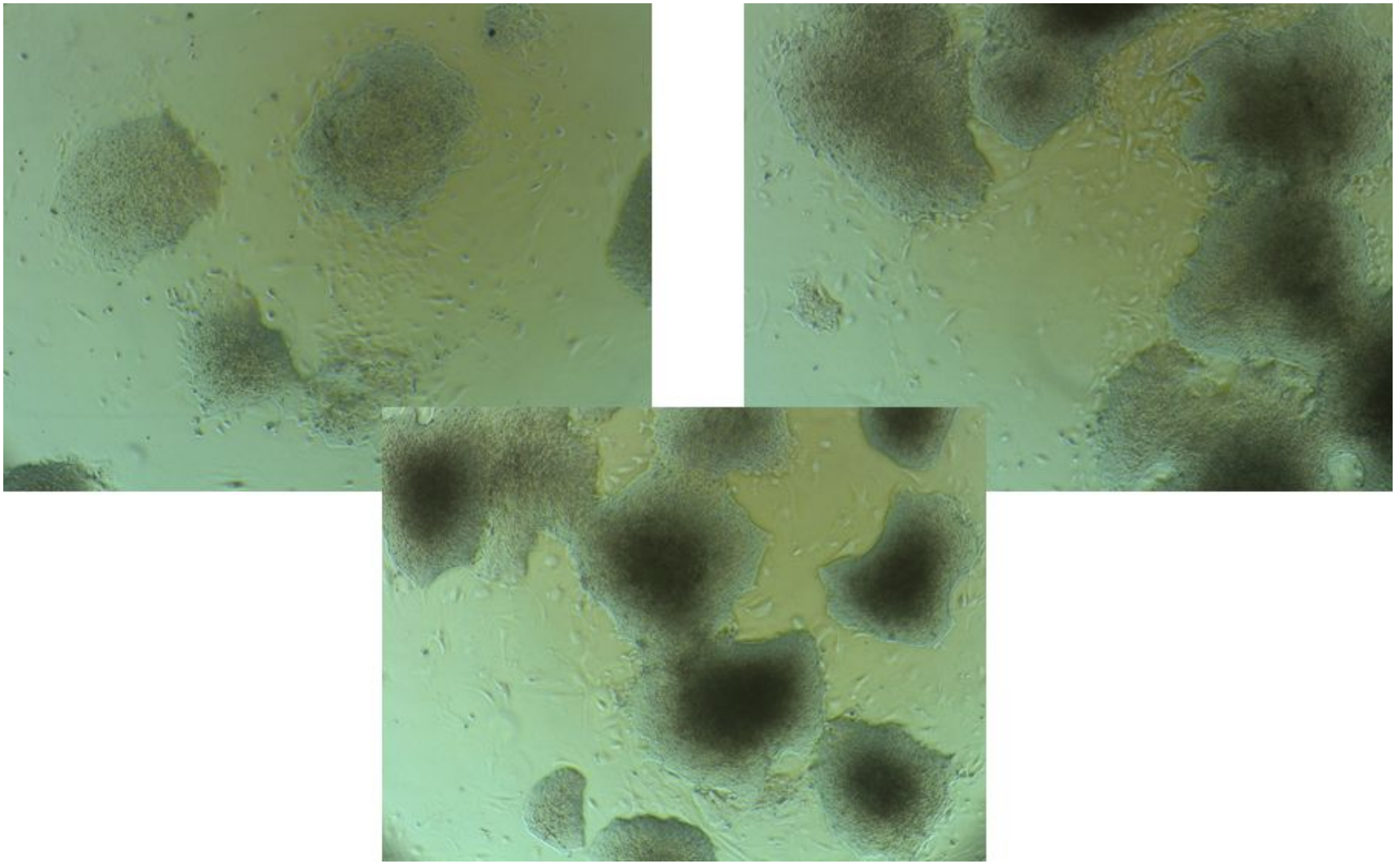
Active Task Status – Past Month

NCSU Safe Genes: Active Task Status - :Dec 2017

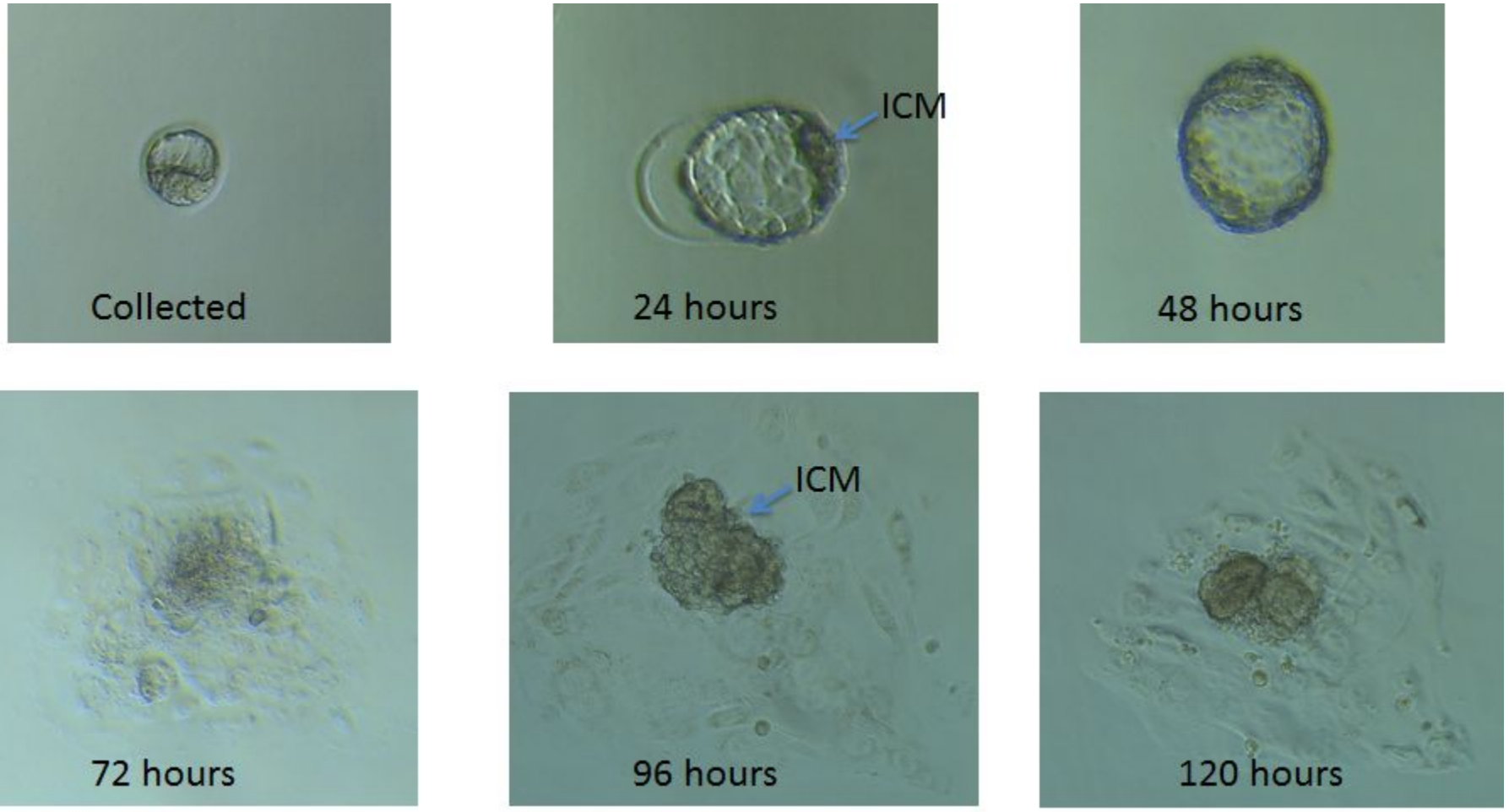
SOW Task #		Contract Start	Due Date	Actual Start	Actual Finish	Status (%)		Exit Criteria (Milestones and Deliverables)	Issues and Status
TA1 – Control of Genome Editing Activity									
3.1.1.1	Engineer t-Sry mice	5/1/2017	2/1/2019	6/1/2017	In progress	22%		Engineer t-Sry mice to express Sry under doxycycline control	iPS Cells passaged 4 times
3.1.1.2	Generation 1 drive mice	5/1/2017	11/30/18	7/1/2017	In progress	24%		Assess stability, efficiency of CAS9 & CPF1-mediated germline and zygotic homing	Zygotic drive mice with CMV promoter screened. Mosaic mice generated, but indel mutations are variable. CPF1 lines generated.
3.1.1.3	Feminizing Y-shredder drive	5/1/2017	2/28/19	1/1/2018	In progress	17%		Develop an efficient feminizing endonuclease gene drive (Y-shredder)	Effective Y-chromosome target identified.
3.1.1.4	Identify Population-specific alleles	5/1/2017	2/28/19	6/30/2017	In progress	20%		Identify population-specific Private Alleles in six mouse island population and adjacent mainland populations and conduct population genetic analyses	Post-doc recruitment initiated. Transport permits for mouse tissue in progress. Organizing collections of Australian mouse tissue.
3.1.1.5	Develop PAM-sensitive gene drive	5/1/2017	4/30/19	Not yet started		0%		Develop efficient PAM-sensitive gene drive	Will utilize inputs from 3.1.1.2-3.1.1.4
3.1.2	Systematic and structured hazard analysis	5/1/2017	2/28/19	Not yet started		0%		Description of Adverse Outcome Pathways	Will utilize inputs from 3.1.1.2 and 3.1.1.3 to initiate analysis
3.1.3	Mathematical modeling of performance of Genome editors	5/1/2017	2/28/19		In progress	20%		Spatial, stochastic individual-based model for mouse population and analysis of gene drive strategies	Post-docs hired in both modeling groups.
3.1.4	Regulatory Engagement	5/1/2017	4/30/2019	5/3/2017	In progress	20%		Analysis and outcomes of the meetings and recommendations for a path forward for gene drives informed by input from regulatory agencies	FDA will be initial US regulatory agency with authority over this project
3.1.5	Stakeholder Engagement	5/1/2017	2/28/2019	9/1/2017	In progress	17%		Draft technology scenarios, Workshop report with recommendations, stakeholder map	Finalized and prioritized stakeholder interview protocol

Task 3.1.1.1 - Engineer t-Sry mice

iPS cells before the 4th passage



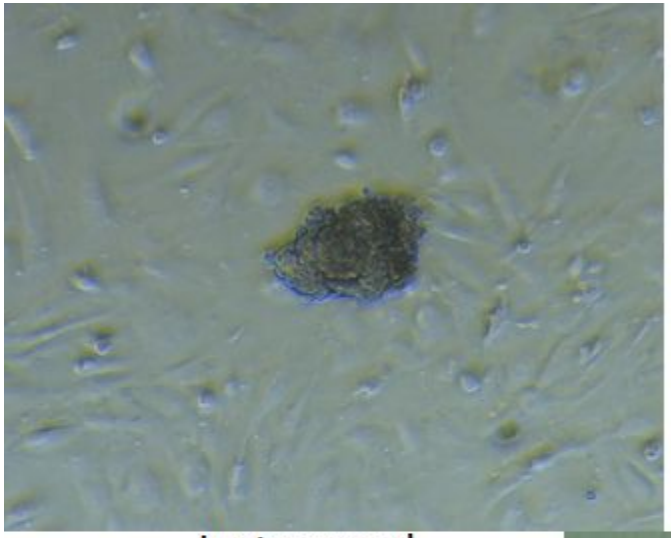
Task 3.1.1.1 - Engineer t-Sry mice



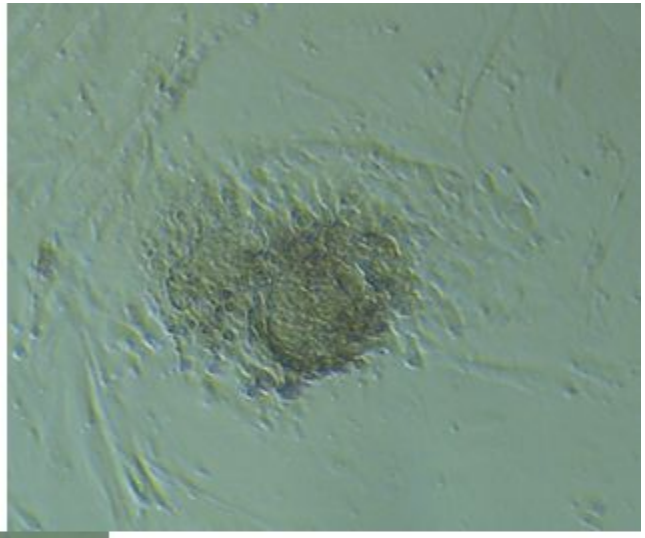
ES cells

- Blastocysts were collected on 3.5 dpc.
- The blastocyst hatches from the zona pellucida and attaches to the plate.
- Trophoblast cells are the large ones that spread out. The inner cell mass (ICM) grows and become the ES cells.

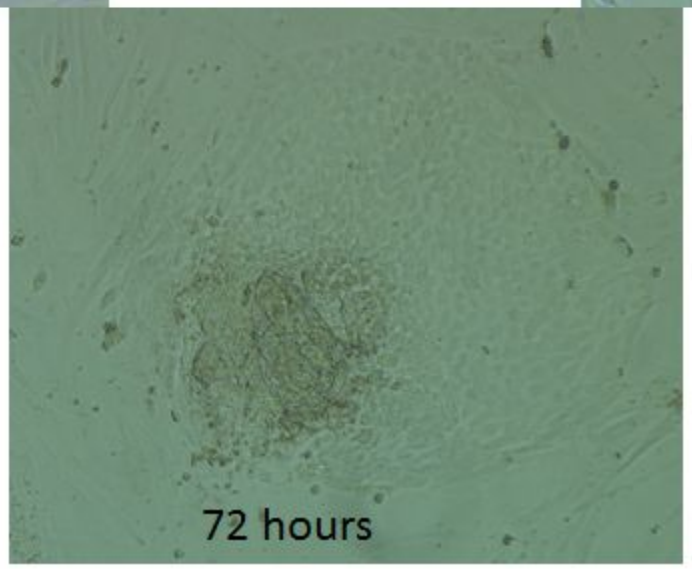
Task 3.1.1.1 - Engineer t-Sry mice



Just moved



24 hours



72 hours

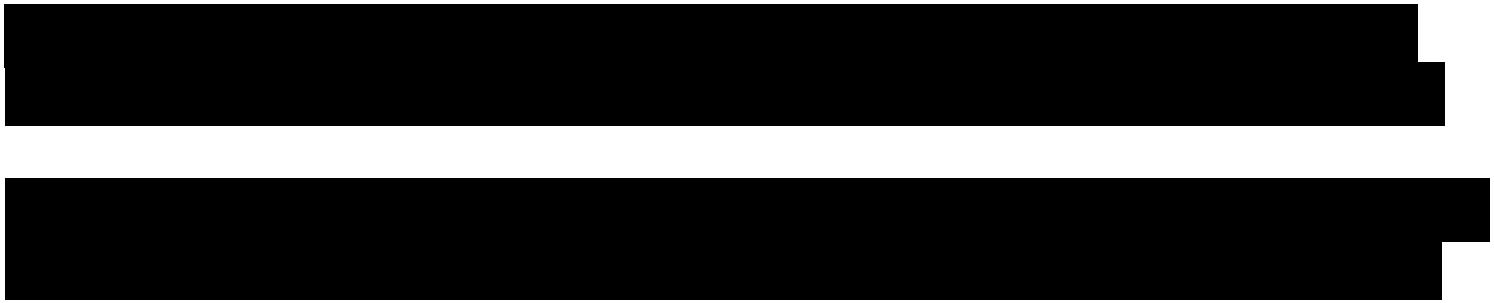
ES cells

- After day 5, the inner cell mass was moved to a new plate

Task 3.1.1.3 - Feminizing Y-Shredder - CRISPR mice

Identified Y chromosome gRNA (41X; Adikusuma et al 2017 *Mol. Therapy*) that shreds Y chromosome efficiently in ES cells

Transient expression in zygotes also results in Y chromosome shredding (Adikusuma et al 2017 *Mol. Therapy*)



Task 3.1.1.4.1 - Island Selection

Obligate Criteria:

1. Island is within a country with a mature regulatory environment for genetically modified organisms. Our primary focus will be on the U.S. and Australia due to having research team members in both countries, but island possibilities in New Zealand will also be considered.
2. Island is within a country or overseas territory where *Mus musculus* populations are present and non-native.
3. Island is biosecure based on two key criteria: i) Either closed to the public or has only infrequent and controlled visitation, ii) Remote enough to avoid unassisted immigration or emigration of mice (i.e., >1km from other land masses).
4. *M. musculus* are the only rodent species present.

Desirable Criteria:

5. Reasonably economical and feasible to visit the island year-round
6. No challenges exist to treatment using traditional, rodenticide-based methods to eradicate mice. Key characteristics include:
   a. Uninhabited (besides research station or similar)
   b. No livestock present
   c. No native rodents
   d. No species endemic to that island that may be negatively impacted by a rodenticide application
   e. No non-target species of concern
   f. Regulatory environment allows the use of brodifacoum bait products
   g. Small - island size <300 ha
   h. Single land managing entity.

Task 3.1.1.4.1 - Island Selection

Island	Regulatory	*Mus* are non-native	Biosecure	*Mus* are only rodent	Logistics	Amenable to rodenticide treatment	Size and Notes
S.E. Farallon Island	✓	✓	✓	✓	✓	Challenging	39 ha, rodenticide eradication effort stalled
Sand Island, Midway	✓	✓	✓	✓	✓	✓	486ha
Thevenard, Direction Is., W.A.	✓	✓	✓	✗	✓	Possible	612 & 24 ha, Forrest's mouse present *(Leggadina forresti)* - not endemic
Boullanger & Whitlock, W.A.	✓	✓	✓	✓	✓	Plans underway for rodenticide treatment	Dibblers and dunnarts present (*Parantechinus apicalis, Sminthopsis boullangerensis)*, 34 ha for Boullanger, 5 ha for Whitlock)
Figure of Eight Is., W.A.	✓	✓	✓	✓	✓	Challenging (Dugites [snake] could ingest mice)	248 ha
Browse Is., W.A.	✓	✓	✓	✓	✓	✓	12 ha (35 acres), need to confirm *Mus* present

Task 3.1.1.4.1 - Island Selection

Island	Status and Notes
S.E. Farallon Island	130 mouse samples collected from SE Farallon, 0 from mainland sources thus far
Sand Island, Midway	50 from Midway, ~55 from Oahu sources
Thevenard, Direction Is., W.A.	Animal Ethics approval for Dept Biodiversity, Conservations and Attractions (DBCA) team, working on ACURO procedures
Boullanger & Whitlock, W.A.	Animal Ethics approval for DBCA team, working on ACURO procedures
Figure of Eight Is., W.A.	Animal Ethics approval obtained for DBCA team, working on ACURO approval
Browse Is., W.A.	Animal Ethics approval for DBCA team, working on ACURO approval
USVI Islands	No updates following hurricanes in September, 2017

Task 3.1.5 - Stakeholder Engagement

- Stakeholder Interview Protocol has been finalized
- List of Stakeholders to be interviewed has been prioritized

Upcoming Tasks

Anticipated work for next reporting period:

- 3.1.1.1: iPS Cells to passage #7, allowing Sendai screening
  -expanding iPS cells and ES cells to get to usable levels
- 3.1.1.2: Complete matings to enable homing cross for Vasa-Cas9 Generation 1 drive
  Off-target analysis of Cento 41X Y shredder gRNA
- 3.1.1.4: Transfer Farallon and Oahu samples, initiate sample preparation
- 3.1.2: Risk Assessment tasks to be initiated.
- 3.1.3: Manuscript on modeling of locally-fixed alleles, continue model development/refinement
- 3.1.4: Meet with Larissa Rudenko of FDA, update on project activities
- 3.1.5: Share prioritized stakeholder list with group of experts and pilot interview protocol

Restoring Ecosystems and Biodiversity through Development of Safe and Effective Gene Drives:
New Tasks in Coming Month

Date: 1/16/2018

SOW Task #		Contract Start	Due Date	Actual Start	Predicted Finish	Status (%)	Exit Criteria (Milestones and Deliverables)	Reason for Delay
3.1.1.1	Engineer t-Sry mice	5/1/2017	2/1/2019	6/1/2017	In progress	22%	Engineer t-Sry mice to express Sry under doxycycline control	Awaiting ACURO approval
3.1.1.2	Generation 1 drive mice	5/1/2017	11/30/18	7/1/2017	In progress	24%	Generation of 6 transgenic lines for Generation 1 homing experiments	N/A.
3.1.1.4	Identify Population-specific alleles	5/1/2017	2/28/19	6/30/2017	2/28/19	20%	Identify population-specific Private Alleles in six mouse island population and adjacent mainland populations	Budgeting and approval process for Australian tissue collections
3.1.3	Mathematical modeling of performance of Genome editors	5/1/2017	2/28/19	6/30/2017	2/28/19	20%	Spatial, stochastic individual-based model for mouse population and analysis of gene drive strategies	N/A
3.1.4	Regulatory Engagement	5/1/2017	4/30/2019	5/3/2017	4/30/2019	20%	Analysis and outcomes of the meetings and recommendations for a path forward for gene drives informed by input from regulatory agencies	N/A
3.1.5	Stakeholder Engagement	5/1/2017	2/28/2019	9/1/2017	4/30/2019	17%	Draft technology scenarios, Workshop report with recommendations, stakeholder map	N/A

Public Affairs and Public Engagement

- Publications
  - Not directly related to *Safe Genes* project, but special issue of *Journal of Responsible Innovation* on gene drives available online now with numerous performers from our team and others
    - see **https://tinyurl.com/y8z93cf6 or http://www.tandfonline.com/toc/tjri20/current**
- Meetings
  - Nothing to report
- Items for Public Release
  - None as yet

Compliance

- Animal Use protocols
  - Acuro approval pending for Texas A&M component of project.
  - Clarification of ACURO protocol for collection of tissue samples by Australian collaborators

Additional Items for Discussion

- FOIA request update

Detailed spend plan