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

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Royden Saah and Karl Campbell - Island Conservation

Project PoP: 5/1/2017-4/30/2021
Reporting Period: 8/9/2017 to 9/10/2017

Briefing Prepared for Renee Wegrzyn

September 19, 2017

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:

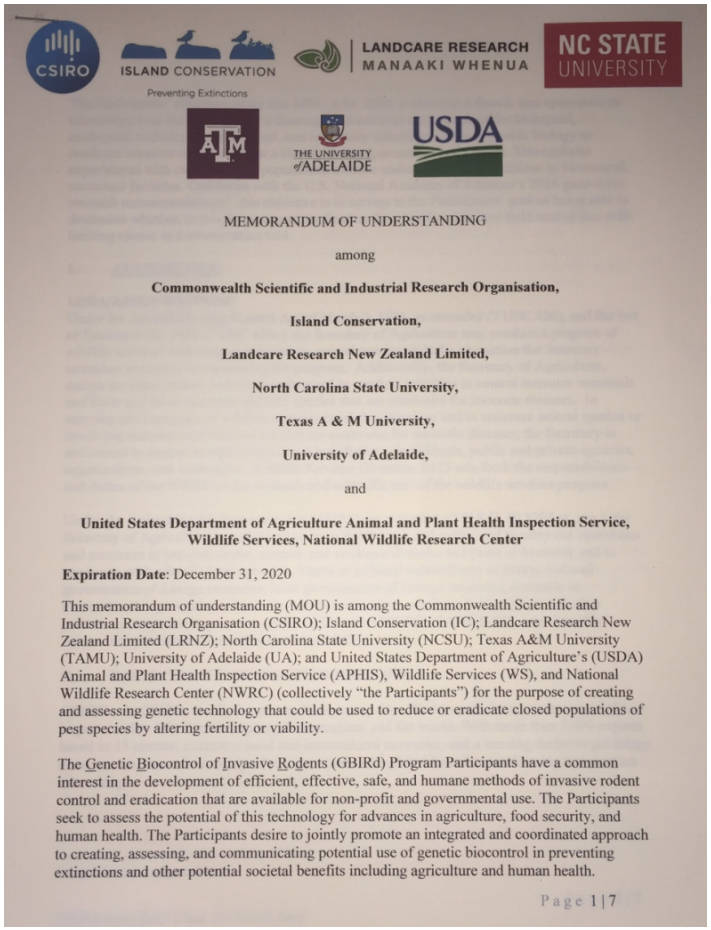
- Develop and test first sex-biasing gene drive mechanisms in mammals including an innovative trans-effector drive
- Identify population specific, locally-fixed genetic targets for gene drive integration to develop and test spatial limitation of gene drive function
- Mathematically model gene drive function 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):

- Multinational GBIRd MoU signed by US, AU, & NZ
- Demonstrated efficient Y-chromosome shredding in ES cells (*Molecular Therapy, 9/17)*
- "Target" founder mice generated & CMV-Cas9 imported and validated

Challenges:



MEMORANDUM OF UNDERSTANDING

among

Commonwealth Scientific and Industrial Research Organisation,

Island Conservation,

Landcare Research New Zealand Limited,

North Carolina State University,

Texas A & M University,

University of Adelaide,

and

United States Department of Agriculture Animal and Plant Health Inspection Service, Wildlife Services, National Wildlife Research Center

Expiration Date: December 31, 2020

This memorandum of understanding (MOU) is among the Commonwealth Scientific and Industrial Research Organisation (CSIRO); Island Conservation (IC); Landcare Research New Zealand Limited (LRNZ); North Carolina State University (NCSU); Texas A&M University (TAMU); University of Adelaide (UA); and United States Department of Agriculture's (USDA) Animal and Plant Health Inspection Service (APHIS), Wildlife Services (WS), and National Wildlife Research Center (NWRC) (collectively "the Participants") for the purpose of creating and assessing genetic technology that could be used to reduce or eradicate closed populations of pest species by altering fertility or viability.

The Genetic Biocontrol of Invasive Rodents (GBIRd) Program Participants have a common interest in the development of efficient, effective, safe, and humane methods of invasive rodent control and eradication that are available for non-profit and governmental use. The Participants seek to assess the potential of this technology for advances in agriculture, food security, and human health. The Participants desire to jointly promote an integrated and coordinated approach to creating, assessing, and communicating potential use of genetic biocontrol in preventing extinctions and other potential societal benefits including agriculture and human health.

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Technical Progress - Executive Overview

Technical progress update:

- Engineering of t-Sry mice (3.1.1.1)
  - gRNAs for cutting t-haplotype screened *in vitro*.
  - Mouse embryonic fibroblasts carrying t^{w2}-haplotype produced
- Generation 1 synthetic drive mouse development (3.1.1.2)
  - Obtained ACURO approval to make mice. "Target" strain founder mice are generated and sequence validated. CMV-Cas9 mice imported and validated. Construct for "gRNA Cas9" mice prepared.
- Y-Shredder (3.1.1.3)
  - Identified gRNAs targeting Y-Chr repeats that result in up to 80% reduction in Y-chr in mES cells (confirmed by FISH and qPCR). Published in *Molecular Therapy.*
- Identification of population-specific, locally-fixed alleles ('Private alleles') (3.1.1.4)
  - Interagency Agreement in place between DARPA and USDA
  - NWRC has internal IACUC approval for receiving tissue samples from islands and conducting private allele work
  - Initial list of 11 potential islands developed that are good fit for criteria:
    - 5 in US territory, 6 in Australia
  - Working on US permitting process to bring in samples from islands
    - In process of hiring Post-doc for the work and establishing a cooperative agreement with Colorado state University to hire this person as USDA has a hiring freeze (challenge as there is 13% overhead we have to lose)

Technical Progress - Executive Overview

Technical progress update:

- Modeling (3.1.3) Continued development of in silico model for Y-shredder
  - Meeting of Modelers from NCSU, University of Adelaide, & USDA, occurred 9-12-17. Planning for best approaches to integrating progress in genetics into modeling efforts.

- Regulatory Engagement (3.1.4)
  - Developing a product support guide & workshop regarding US regulatory system for Gene Drive Development for Invasive Species management.
  - Calls with Laura Epstein, Ritu Nalubola (FDA), Stephanie James (FNIH), and others re workshop efforts
  - Meeting with David Threadgill regarding regulatory environment of *Mus musculus* from PI perspective

- Stakeholder Engagement (3.1.5): Landscape Analysis
  - Compiling reference materials for background
  - Delborne attended "Engineering Resilience" workshop (co-sponsored by CSIRO and Revive & Restore). Travel costs not charged to this project.

Milestones and Task Status Overview

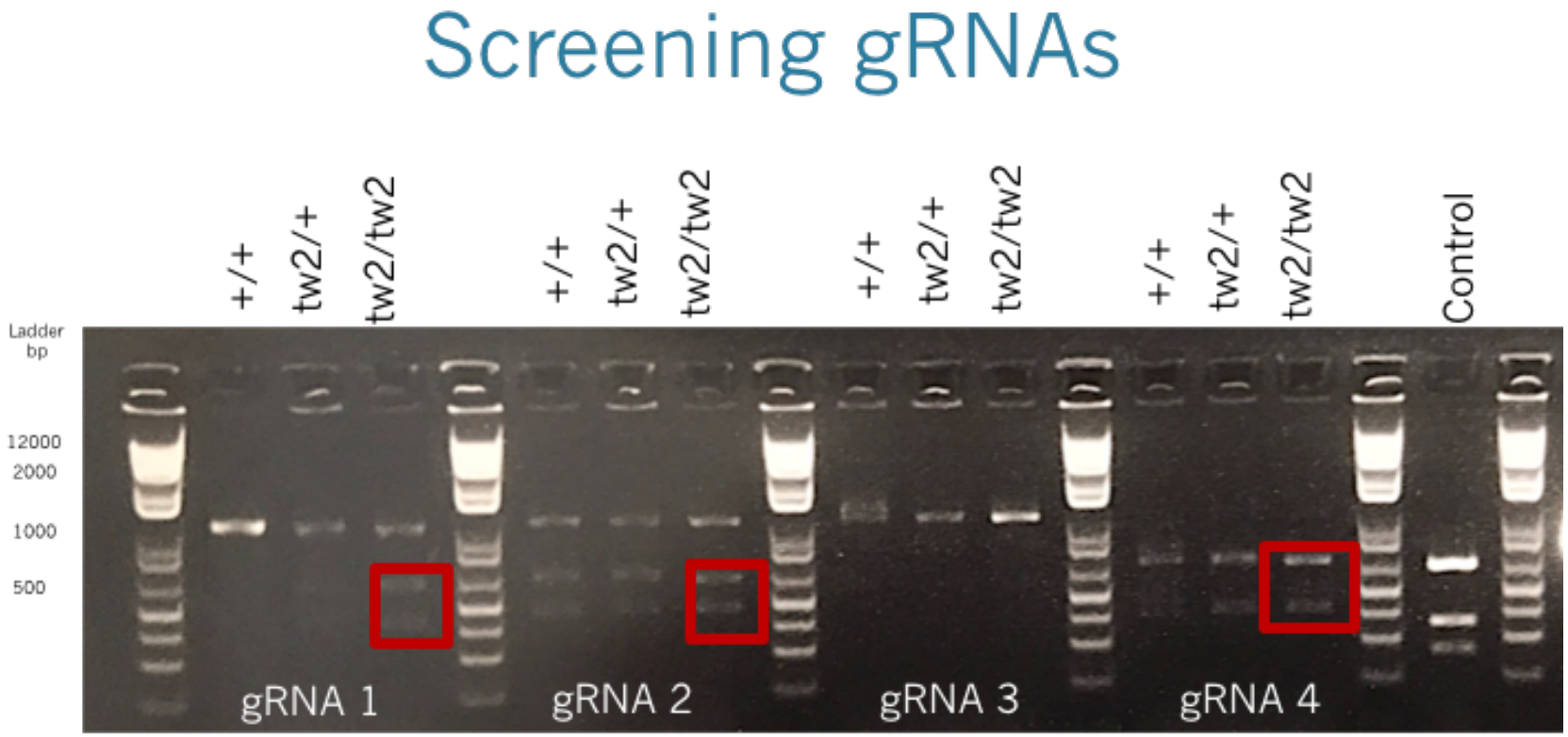
Restoring Ecosystems and Biodiversity through Development of Safe and Effective Gene Drives

Active Task Status – Past Month

Date: 8/10/2017

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	15%	Engineer t-Sry mice to express Sry under doxycycline control	Testing editing approach in cell system, developing IPSCs
3.1.1.2	Generation 1 drive mice	5/1/2017	11/30/18	7/1/2017	In progress	10%	Assess stability, efficiency of CAS9-mediated germline and zygotic homing	ACURO approval obtained "Target" founder mice generated CMV-Cas9 mice imported and validated
3.1.1.3	Feminizing Y-shredder drive	5/1/2017	2/28/19	1/1/2018	In progress	15%	Develop an efficient feminizing endonuclease gene drive (Y-shredder)	Effective Y-shredding achieved in vitro (qPCR and FISH) Publication in *Mol. Therapy.*
3.1.1.4	Identify Population-specific alleles	5/1/2017	2/28/19	6/30/2017	In progress	10%	Identify population-specific Private Alleles in six mouse island population and adjacent mainland populations	Island selection in progress; Contract established with USDA-NWRC; Hurricane effects in USVI
3.11.5	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	10%	Spatial, stochastic individual-based model for mouse population and analysis of gene drive strategies	Building on approaches developed in modeling paper published 8/8/17
3.1.4	Regulatory Engagement	5/1/2017	4/30/2019	5/3/2017	In progress	8%	Analysis and outcomes of the meetings and recommendations for a path forward for gene drives informed by input from regulatory agencies	Awaiting determination of regulatory responsibility determination from US agencies
3.1.5	Stakeholder Engagement	5/1/2017	2/28/2019	9/1/2017	In progress	10%	Draft technology scenarios, Workshop report with recommendations, stakeholder map	Landscape analysis underway

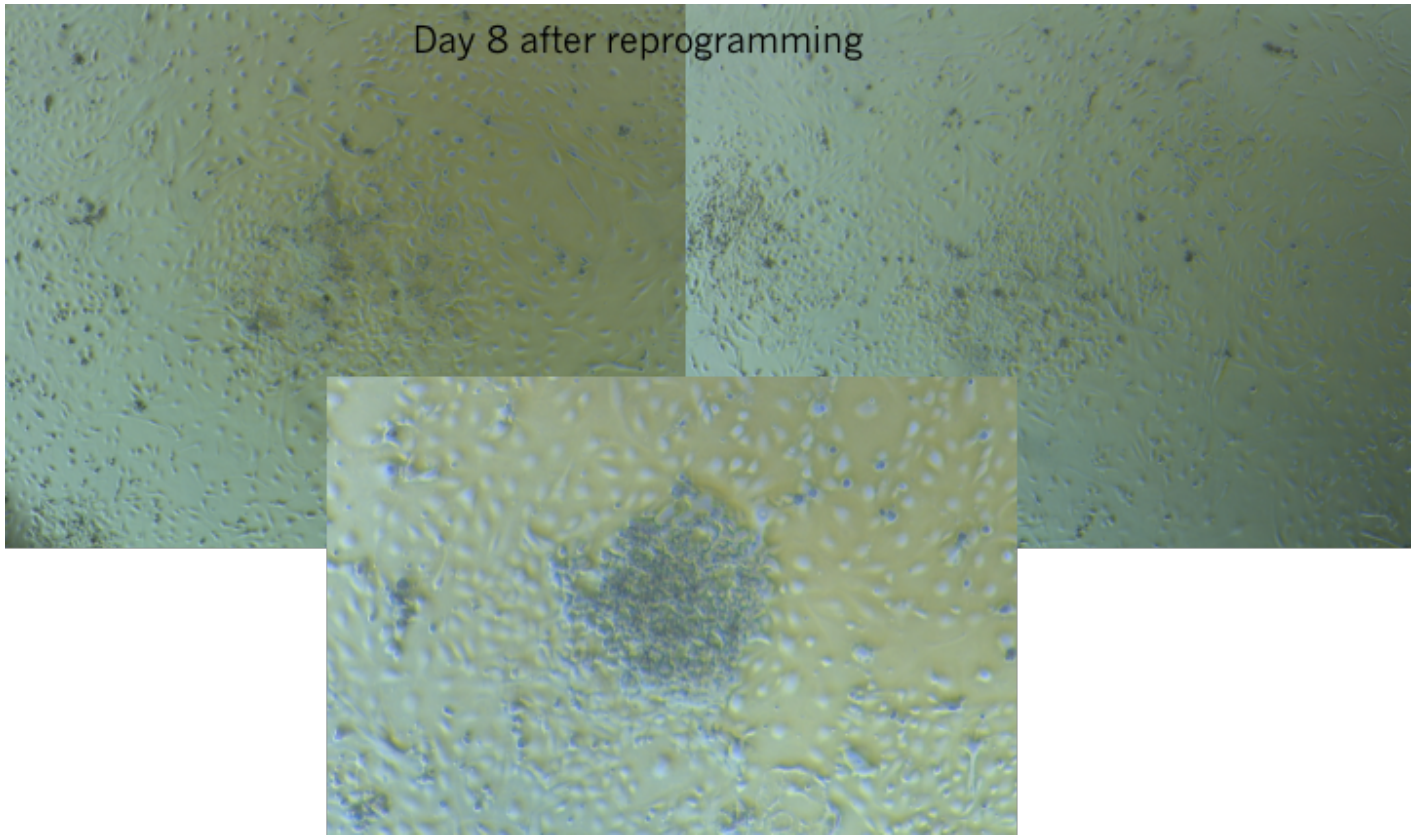
Task 3.1.1.1 - Engineer t-Sry mice



Screening of gRNAs for cutting t-haplotype with wild-type (+/+), heterozygote (tw2/+), and homozygote (tw2/tw2) mouse DNA. gRNA1 cut only the t, gRNA2 and 4 cut wild-type and the t-haplotype, and gRNA3 did not cut effectively.

Task 3.1.1.1 - Engineer t-Sry mice

Reprogramming MEFs

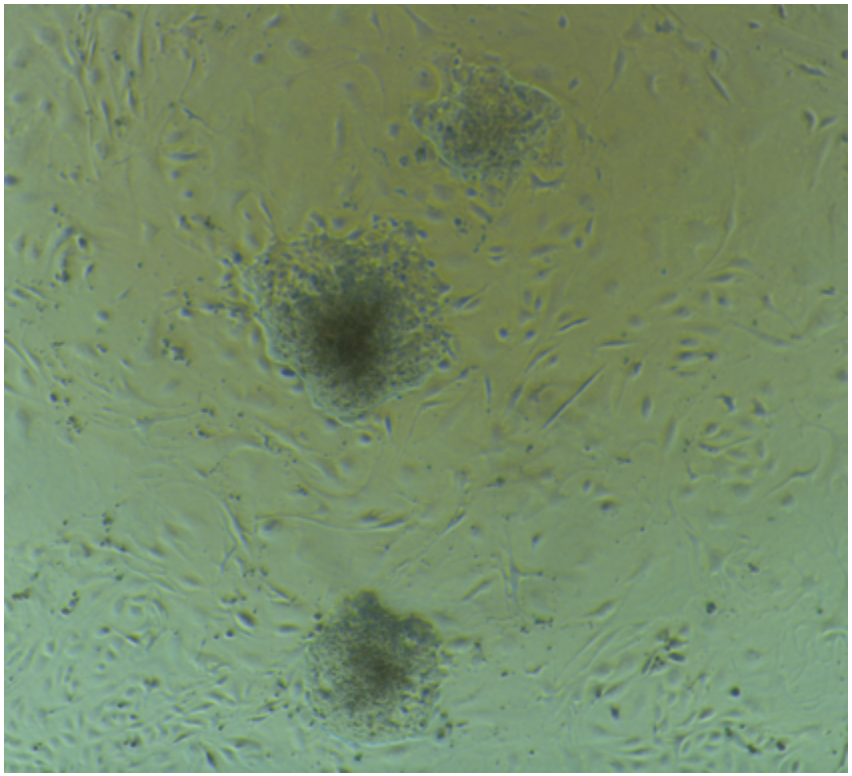


t-haplotype MEFs were reprogrammed with Yamanaka factors, Oct4, Sox2, cMyc, and Klf4, using a Sendai virus. Picture ws taken 8 days after the transfection and before they were passaged for the first time

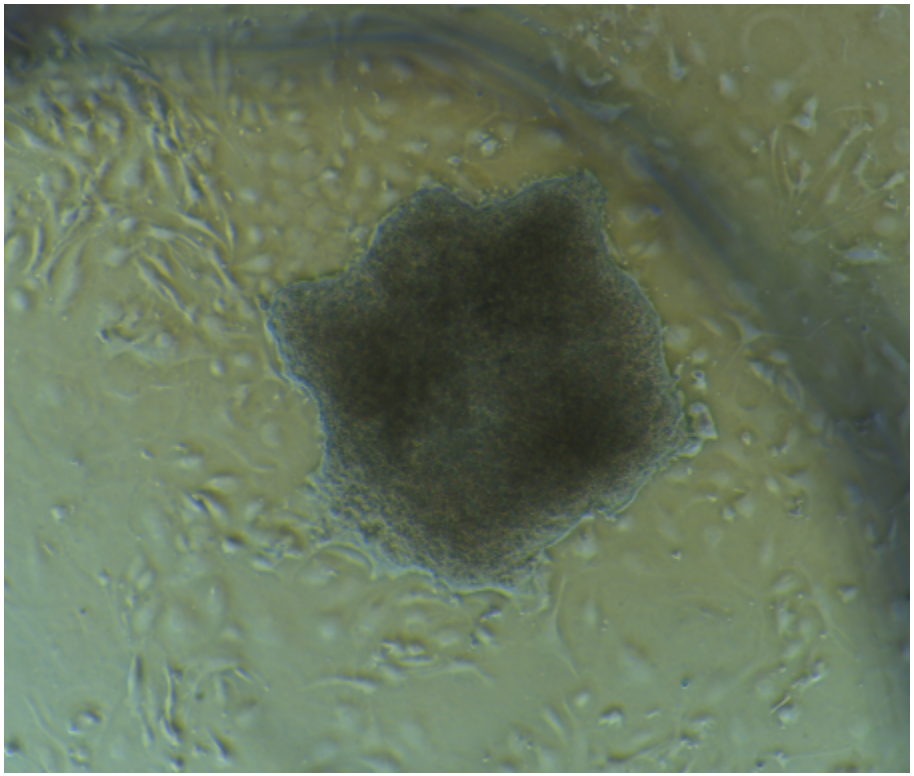
Task 3.1.1.1 - Engineer t-Sry mice

IPSC formation

Day 30 after reprogramming, first subcloning

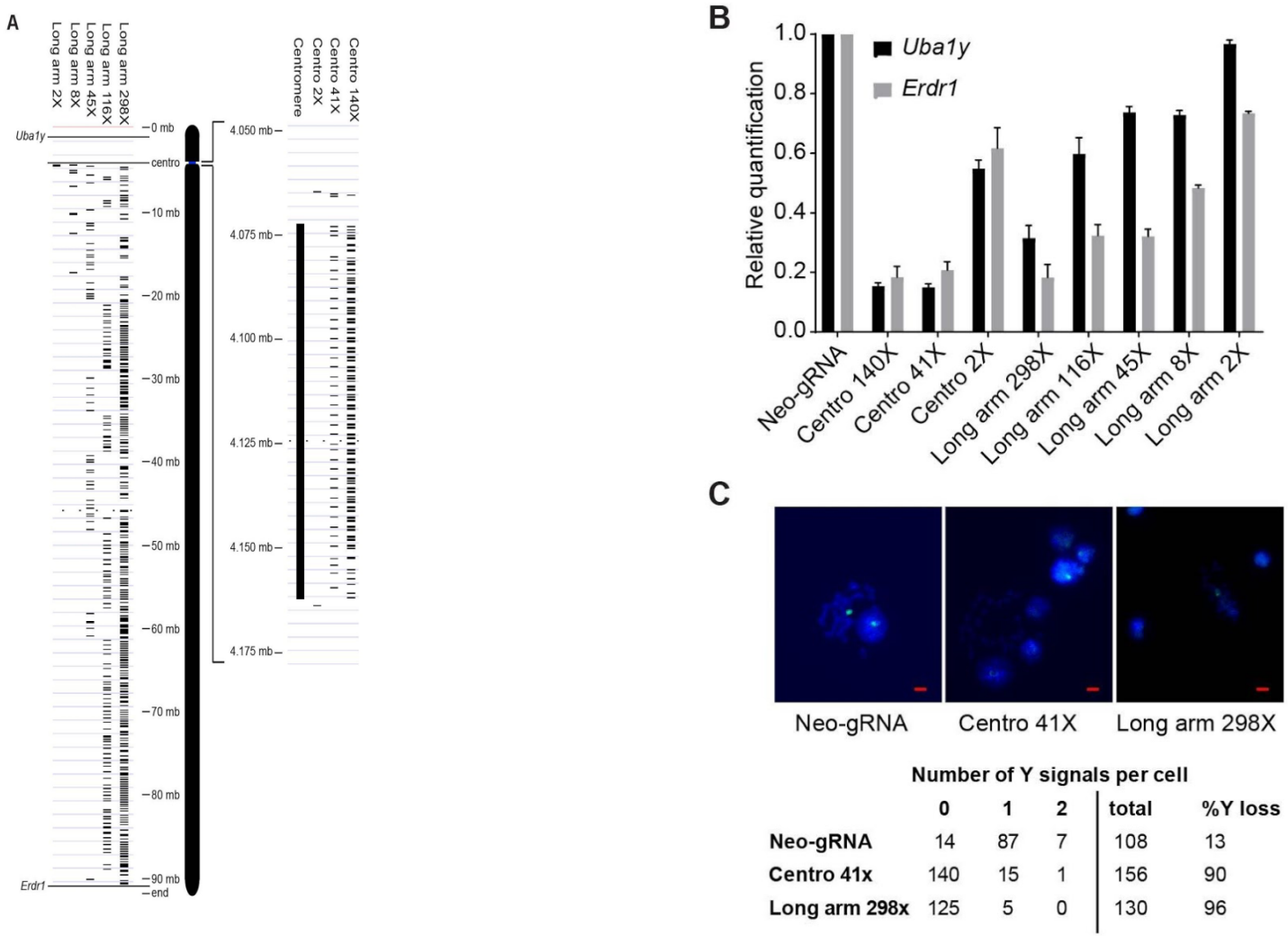


Day 43 after reprogramming, second subcloning



Colonies (6) taken and moved to separate plates in both first and second subclonings

Task 3.1.1.3 (Y-shredder optimization)



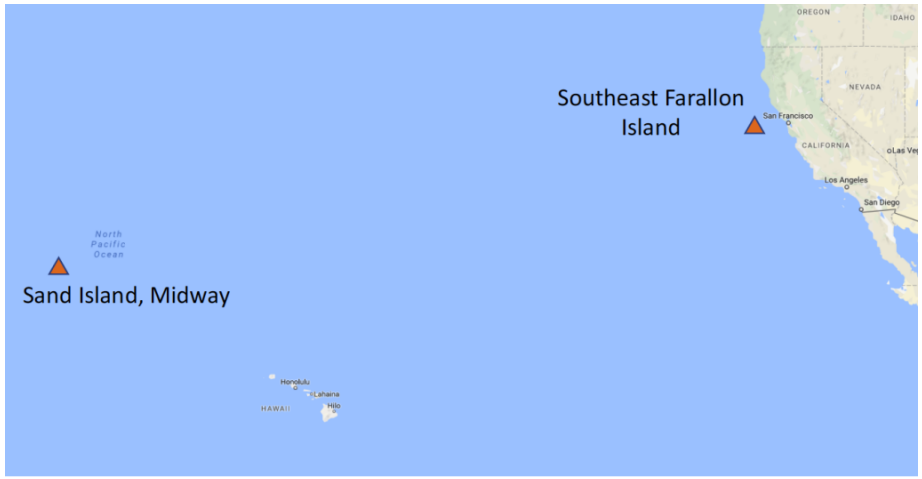
Number of Y signals per cell

	0	1	2	total	%Y loss
Neo-gRNA	14	87	7	108	13
Centro 41x	140	15	1	156	90
Long arm 298x	125	5	0	130	96

(A) Schematic showing position of gRNA target sites in the long arm and centromere of the Y chromosome. (**B**) qPCR of genomic DNA to quantify Y chromosome dosage. *Sox1* qPCR was used as the internal reference control. Data were presented as mean ± SEM from n ≥ 3 biological replicates. (**C**) FISH analysis detection of Y chromosome loss. Y chromosome and DAPI staining was indicated by green and blue signals, respectively. Scale bar = 5 µm. Published in Molecular Therapy (Mol Ther. 2017 Aug 2;25(8):1736-1738.)

Task 3.1.1.4.1 - Island Selection

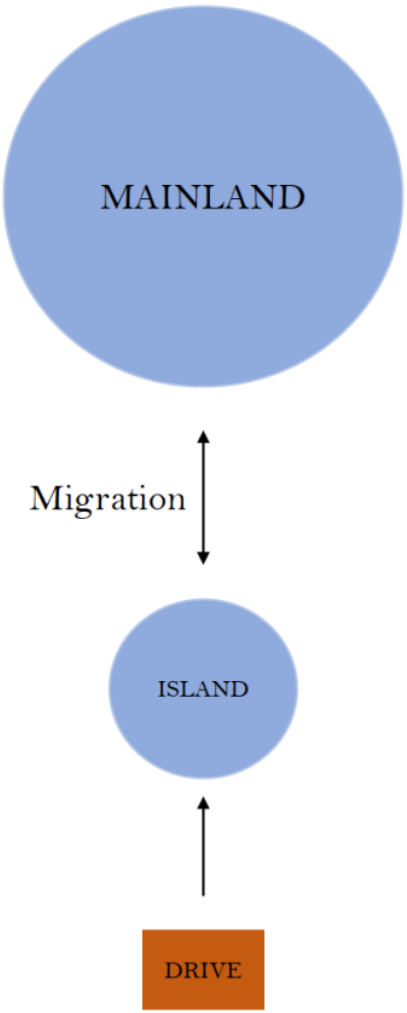
- US Islands
  - Farallon Islands as specified in SOW (confirmed)
  - Sand Island, Midway Atoll: possible
    - Fits obligate criteria well and presence of damaging invasive mouse population confirmed, but slightly over "<300 hectare" size in desirable criteria list
    - Logistically tractable both at Midway and for potential source population (Oahu)
  - US Virgin Islands (Buck, Capella, Saba, Dutch Cap islands): possible
    - Fit obligate and desirable criteria well
    - Logistically tractable in terms of on-site partners with USDA-APHIS and USVI government
    - Uncertainties:
      - Mouse presence likely, but not confirmed
      - Logistical challenges in wake of Hurricane Irma *(potentially also Hurricane Maria now – both Category 5 storms in USVI)*



Task 3.1.1.4.1 - Island Selection

- Identification of population-specific, locally-fixed alleles, Island Selection (3.1.1.4.1) continued: Australian Islands
  - Collaborative relationship established with Department of Parks and Wildlife, Govt of Western Australia through Dr. Margaret Byrne (Director of Science and Conservation)
  - Potential island sampling sites identified through interactions with DPAW personnel
    - Browse, Thevenard, Direction, Boullanger, Whitlock, and Figure of Eight islands
    - Mouse presence likely, but would need confirmation on Browse and Direction Island

Task 3.1.3 - Modeling



Locally Fixed Alleles

DRIVE:

- Suppression- high fitness costs
  - Will also consider sex biasing drive
- Gene drive targets a target susceptible allele
- There is another allele present that cannot be edited by the drive

SETUP:

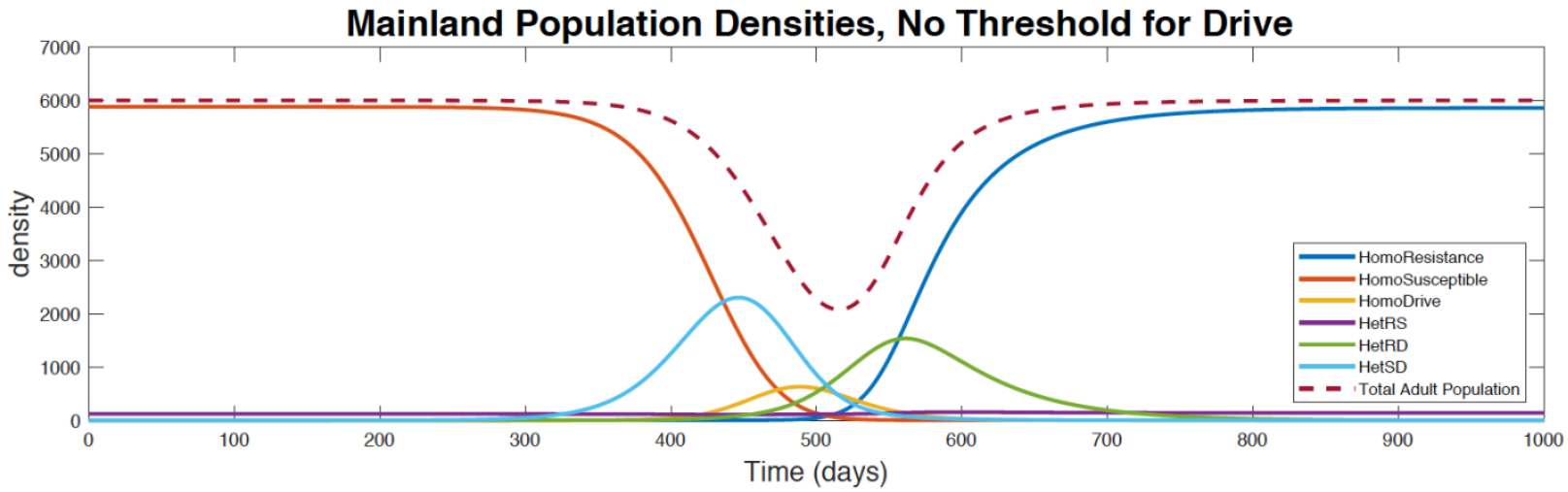
Island and Mainland

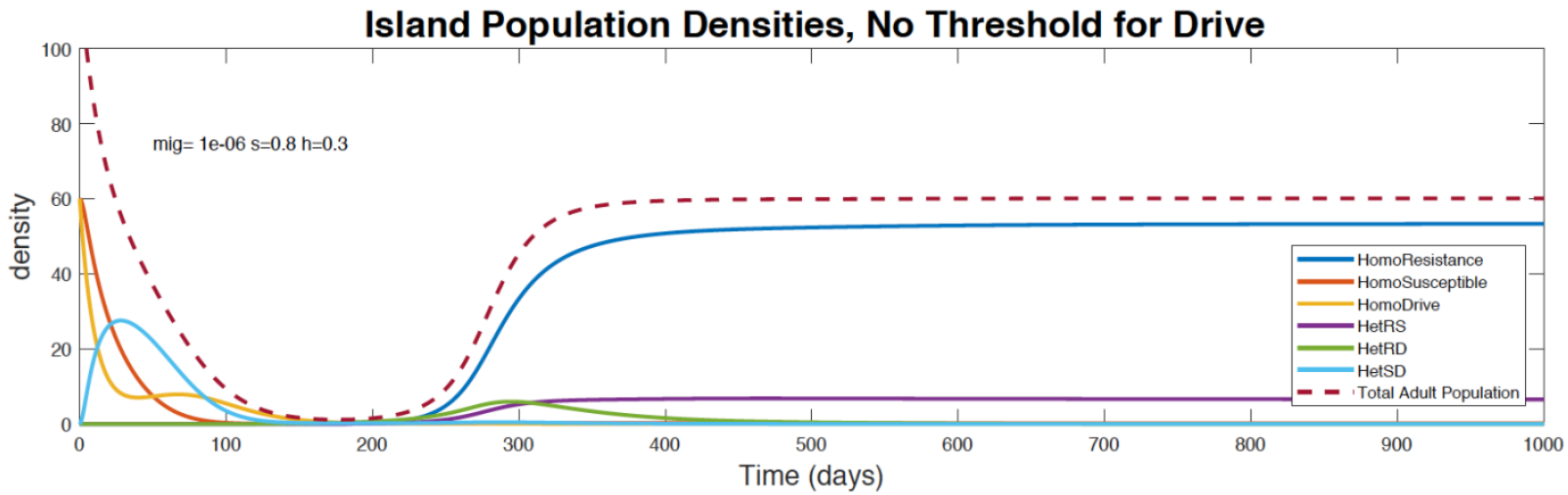
- Island is fixed for target allele
- Mainland has low frequency (< 5%) of resistantance
- Low level of gene flow between island and mainland

QUESTIONS OF INTEREST:

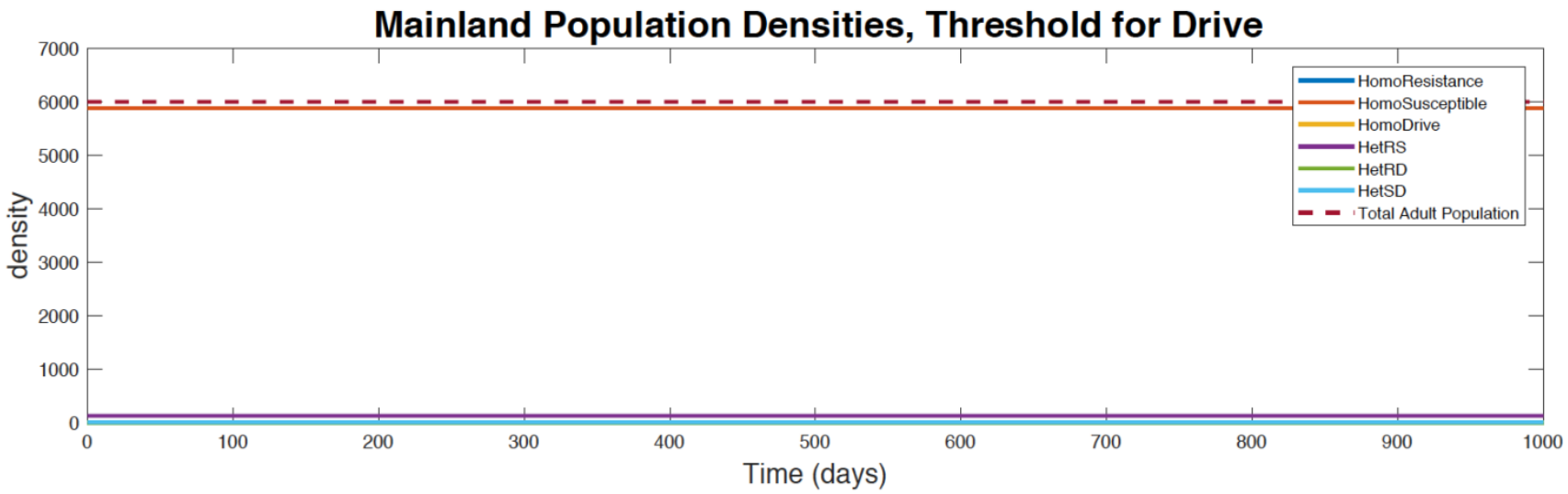
- What is the potential impact of accidental introduction of the drive to the mainland population?
- What prevalence of the resistant gene is necessary to rescue the mainland population upon accidental introduction of the drive?
- Will influx of resistance from the Mainland prevent suppression the island population?

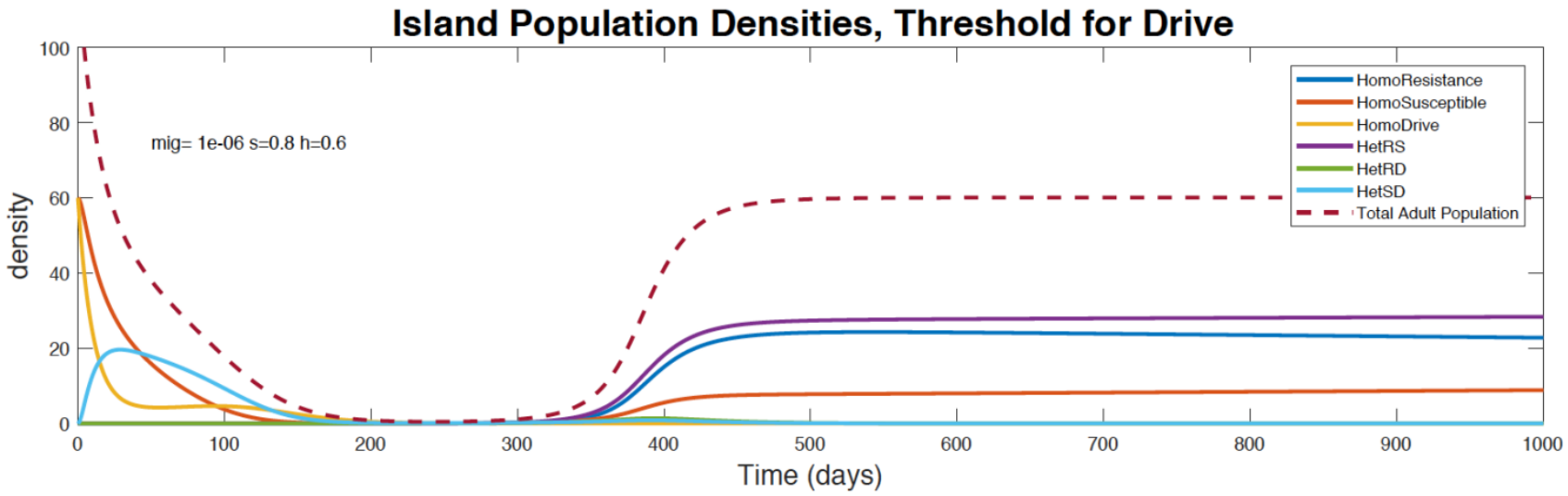
Task 3.1.3 - Modeling





Task 3.1.3 - Modeling





Upcoming Tasks

Anticipated work for next reporting period:

- 3.1.1.1: Testing gRNA cutting efficiency in cells (mouse embryonic fibroblasts); continued development of tw2-carrying IPSCs
- 3.1.1.2: Generation/expansion of GM mouse lines for homing analysis.
- 3.1.1.4: Narrow candidate island list and continue approvals process for collections
- 3.1.3: Continue building on approaches developed in Proc. Royal Society paper (Prowse et al., 2017) and development of a two-patch model to assess locally-fixed allele approach
- 3.1.4: Continue development of a product support guide & workshop regarding US regulatory system for Gene Drive Development for Invasive Species management
- 3.1.5: Identify initial stakeholders for landscape analysis, Generate protocol for outreach to stakeholders

Restoring Ecosystems and Biodiversity through Development of Safe and Effective Gene Drives: Active Task Status – Past month

New Tasks in Coming Month

Date: 9/19/2017

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	15%	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	10%	Generation of 6 transgenic lines for Generation 1 homing experiments	Contract pending between UA and NCSU.
3.1.1.4	Identify Population-specific alleles	5/1/2017	2/28/19	6/30/2017	2/28/19	10%	Identify population-specific Private Alleles in six mouse island population and adjacent mainland populations	Island selection process is on track, challenges with field locations due to hurricanes
3.1.3	Mathematical modeling of performance of Genome editors	5/1/2017	2/28/19	6/30/2014	2/28/19	10%	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	10%	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	5%	Draft technology scenarios, Workshop report with recommendations, stakeholder map	N/A

Public Affairs and Public Engagement

- Publications
  - *Peer-reviewed publications - relevant to project, with partial overlap with Safe Genes:* Adikusuma et al. (2017) Targeted deletion of an entire chromosome using CRISPR/Cas9 *Mol Ther.* 2017 Aug 2;25(8):1736-1738.
  - *Related to Safe Genes, but not supported by project:* Vella et al. (2017) Evaluating strategies for reversing CRISPR-Cas9 gene drives. *Scientific Reports* Sep 8;7(1):11038. doi: 10.1038/s41598-017-10633-2.

- Meetings
  - Engineering Resilience Workshop (Heron Island, Australia) - attendees included Campbell*, Delborne*, Edwards*, Thomas. (*made presentation)

- Public Engagement/Outreach
  - Upcoming: Godwin and Delborne, Science Cafe at North Carolina Museum of Natural Sciences, Raleigh, NC on 9/27/2017

- Items for Public Release
  - None as yet

Compliance

- Animal Use protocols
  - Institutional Animal care and use committees: USDA-NWRC (Already approved for NCSU, University of Adelaide, Texas A&M)
  - HSR: Not applicable and IRB review not required, documentation provided to DARPA from NCSU IRB Coordinator.

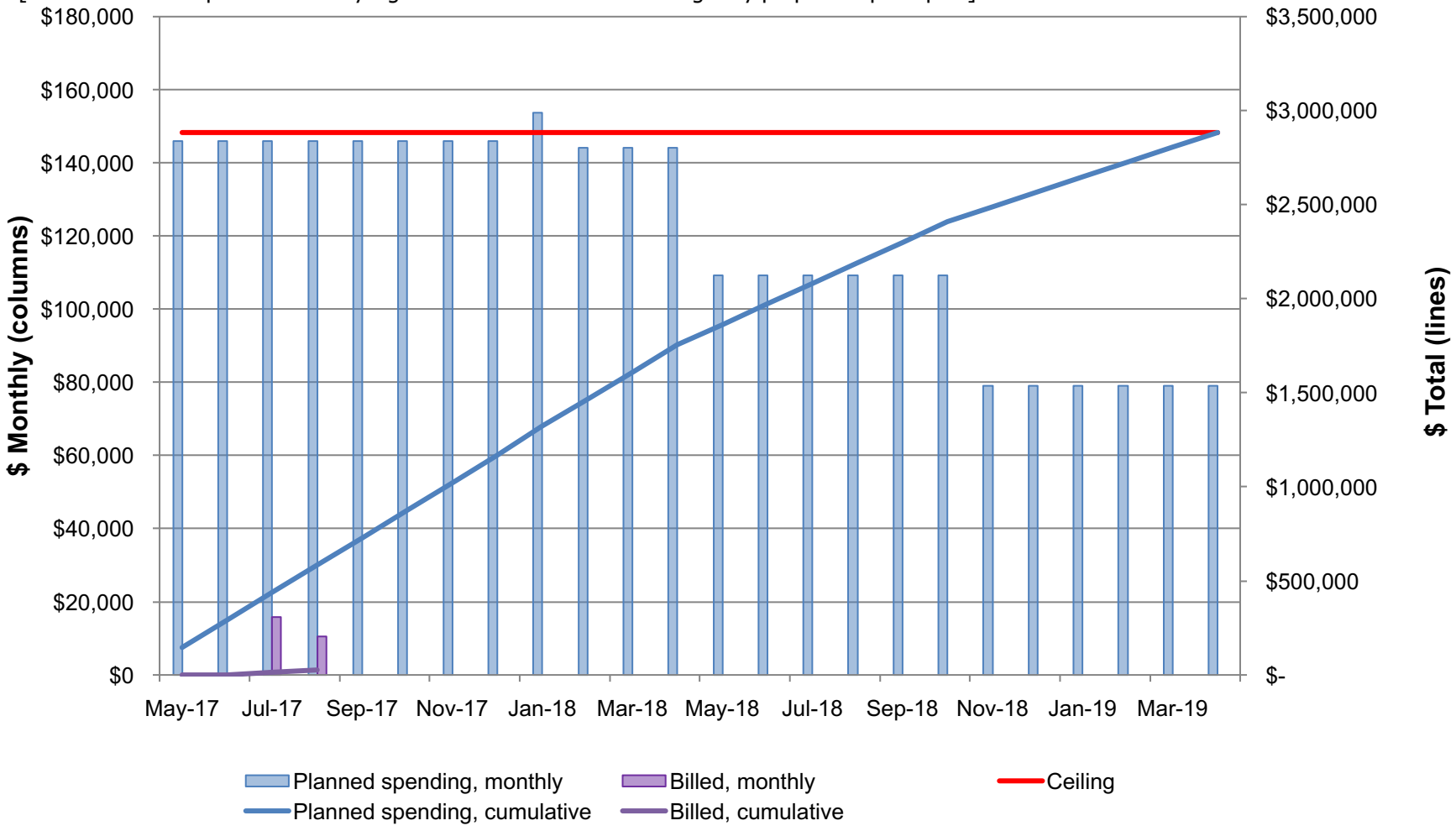
Additional Items for Discussion

- Administrative
  - Change in increment structure in mid-September - may necessitate some minor revisiting of contracts

- Consideration/Approval of SOW changes and process for this?
  - Technical approach for locally-fixed alleles component of project
  - Outdoor enclosures to test approaches in Phase I using *non-transgenic* and unmodified mice?

Detailed spend plan

- Financials: [Indicate original spend plan in contract, percent of funds expended, balance relative to spend plan, funding issues, cost risks]
- [Add financial information to the embedded chart—data included now is just to be used as an example]
- [Provide a short explanation of any significant deviation from the originally proposed spend plan]



Spend Plan Deviation Details/Mitigation plan

- Subcontracting process and fully executing these has slowed invoicing, but we are progressing in this area