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: [10/11/17-11/6/17]

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:

- First evidence of a potential synthetic gene drive in a vertebrate (mammal-mouse)
- Regulatory engagement progress brings representatives from all three regulatory agencies to see very early progress and project orientation of NCSU Safe Gene team in a transparent manner
- 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

Challenges:

Technical Progress - Executive Overview

Technical progress update:

- Engineering of t-Sry mice (3.1.1.1)
  - IPSCs are in third passage, soon to be passed again. Initial genotyping and sexing of IPSC colonies done, will be repeated on passage 3 cells after next subcloning
  - MEFs have been made from individual embryos that have been sexed and genotyped.
- Modeling (3.1.3)
  - Initial Models being developed
- Regulatory Engagement (3.1.4)
  - Regulatory Representatives from EPA, FDA, and USDA invited to NCSU Safe Genes meeting - and attended - for awareness of our technical and LEEDR progress.
- Stakeholder Engagement (3.1.5)
  - Questionnaire drafted

Milestones and Task Status Overview

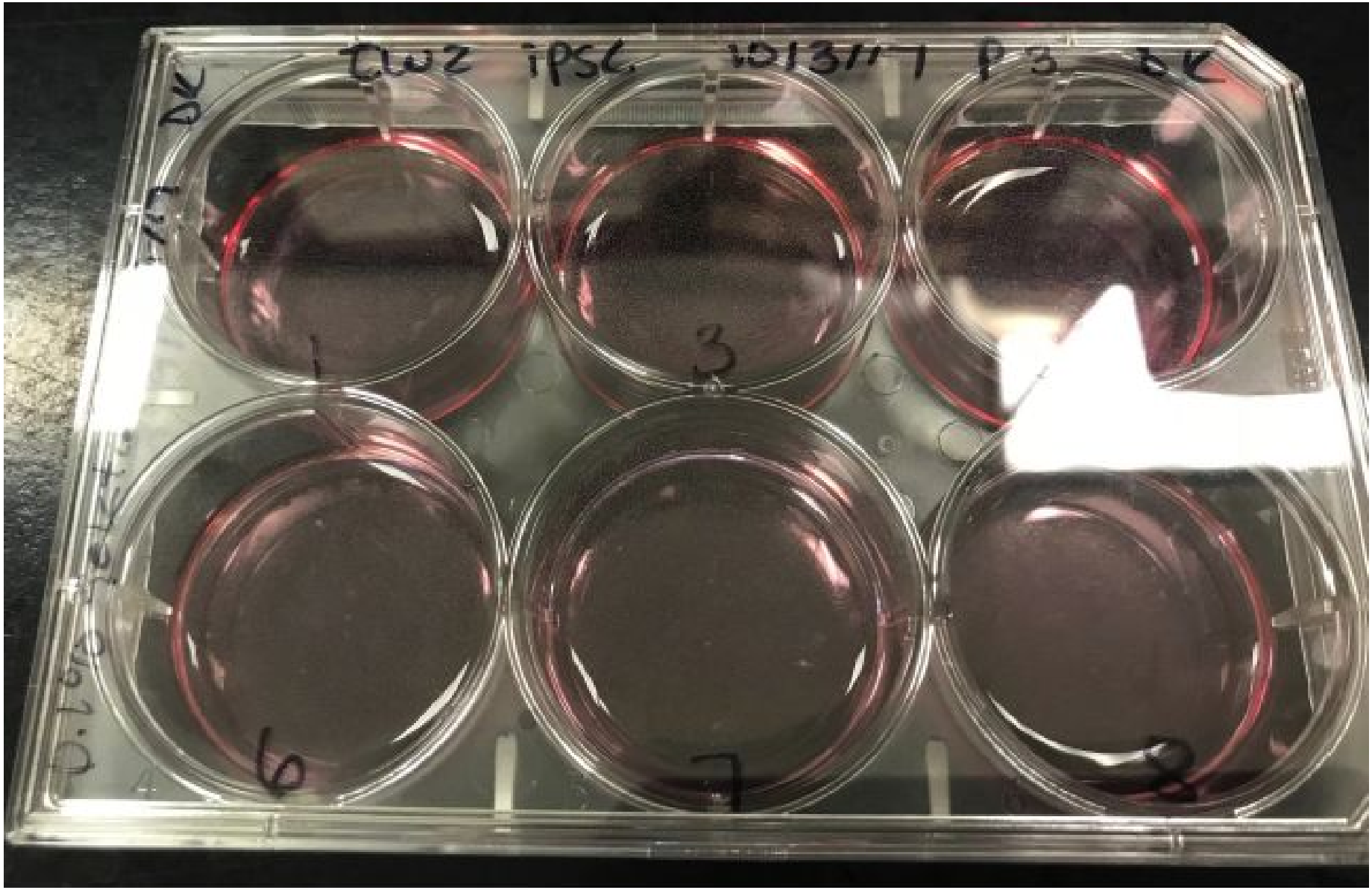
Restoring Ecosystems and Biodiversity through Development of Safe and Effective Gene Drives

Active Task Status – Past Month

Date: 10Dec17

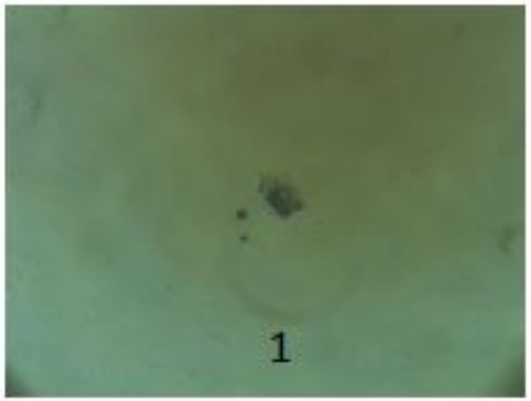
SOW Task #		Contract Start	Due Date	Actual Start	Actual Finish	Status (%)	Exit Criteria (Milestones and Deliverables)	Issues and Status
TA1 – Control of Genome Editing Activity								
3.1.1.1	Engineer t-Sry mice	5/1/2017	2/1/2019	6/1/2017	In progress	17%	Engineer t-Sry mice to express Sry under doxycycline control	Continue generating iPSCs
3.1.1.2	Generation 1 drive mice	5/1/2017	11/30/18	7/1/2017	In progress	17%	Assess stability, efficiency of CAS9-mediated germline and zygotic homing	Validate Vasa-Cas9 expression (qPCR) Create Vasa-Cpf1 founders
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 progress being achieved
3.1.1.4	Identify Population-specific alleles	5/1/2017	2/28/19	6/30/2017	In progress	12%	Identify population-specific Private Alleles in six mouse island population and adjacent mainland populations and conduct populaion genetic analyses	Coordinate samples collection and transfer from Farallon Islands and Midway.
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	13%	Spatial, stochastic individual-based model for mouse population and analysis of gene drive strategies	In Person meeting planned for Co-PIs from NCSU and UofA in Raleigh NC in Dec
3.1.4	Regulatory Engagement	5/1/2017	4/30/2019	5/3/2017	In progress	16%	Analysis and outcomes of the meetings and recommendations for a path forward for gene drives informed by input from regulatory agencies	Productive engagements with all US regulatory entities (i.e. FDA, EPA, and USDA) - attendance for NCSU Safe Genes meeting
3.1.5	Stakeholder Engagement	5/1/2017	2/28/2019	9/1/2017	In progress	12%	Draft technology scenarios, Workshop report with recommendations, stakeholder map	Stakeholder engagement and Landscape analysis initiated

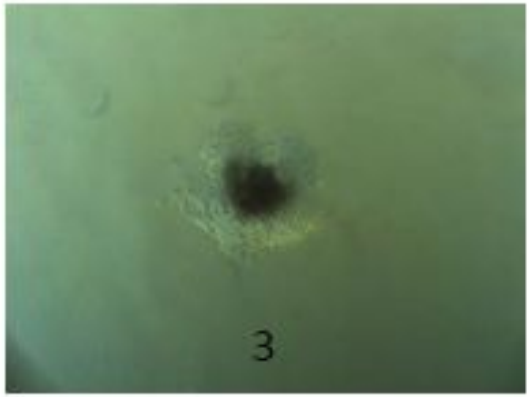
Task 3.1.1.1 - Engineer t-Sry mice

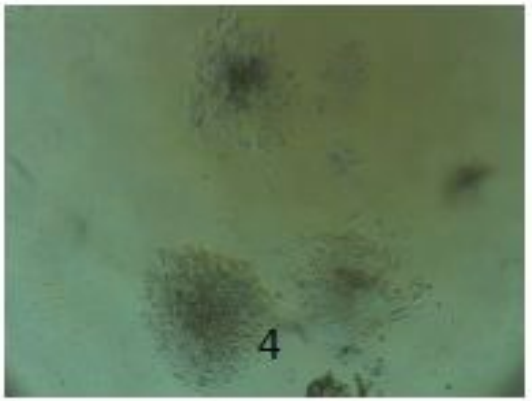


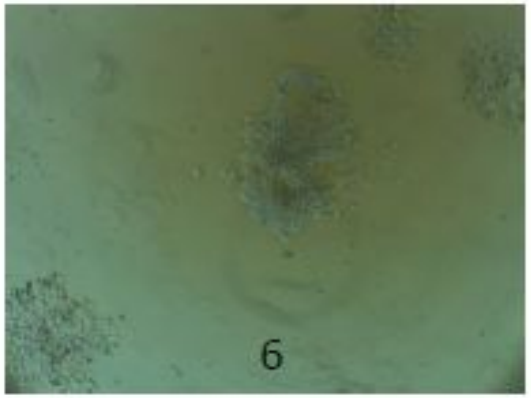
Task 3.1.1.1 - Engineer t-Sry mice

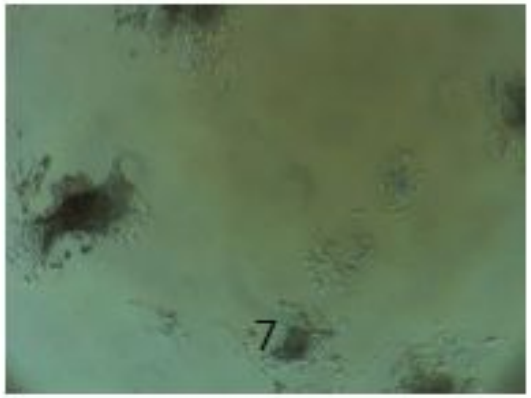
iPSC

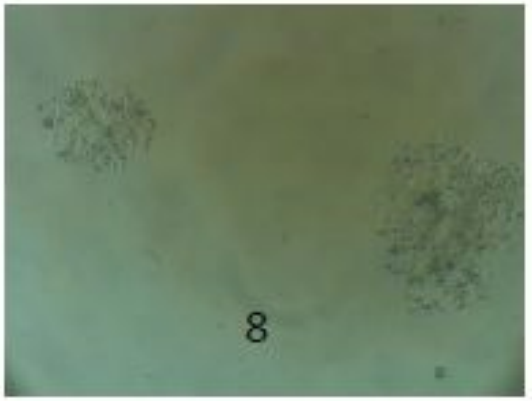










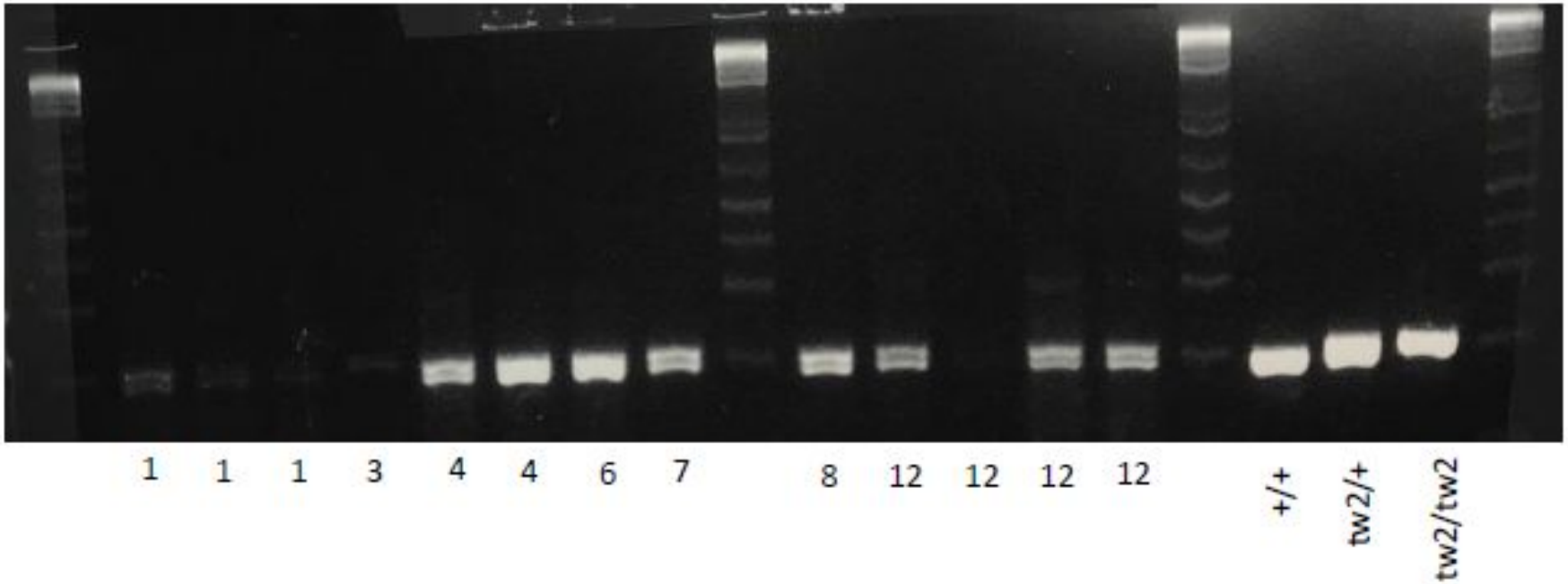


Task 3.1.1.1 - Engineer t-Sry mice

iPSC Genotyping

tw2 genotyping of iPSC colonies

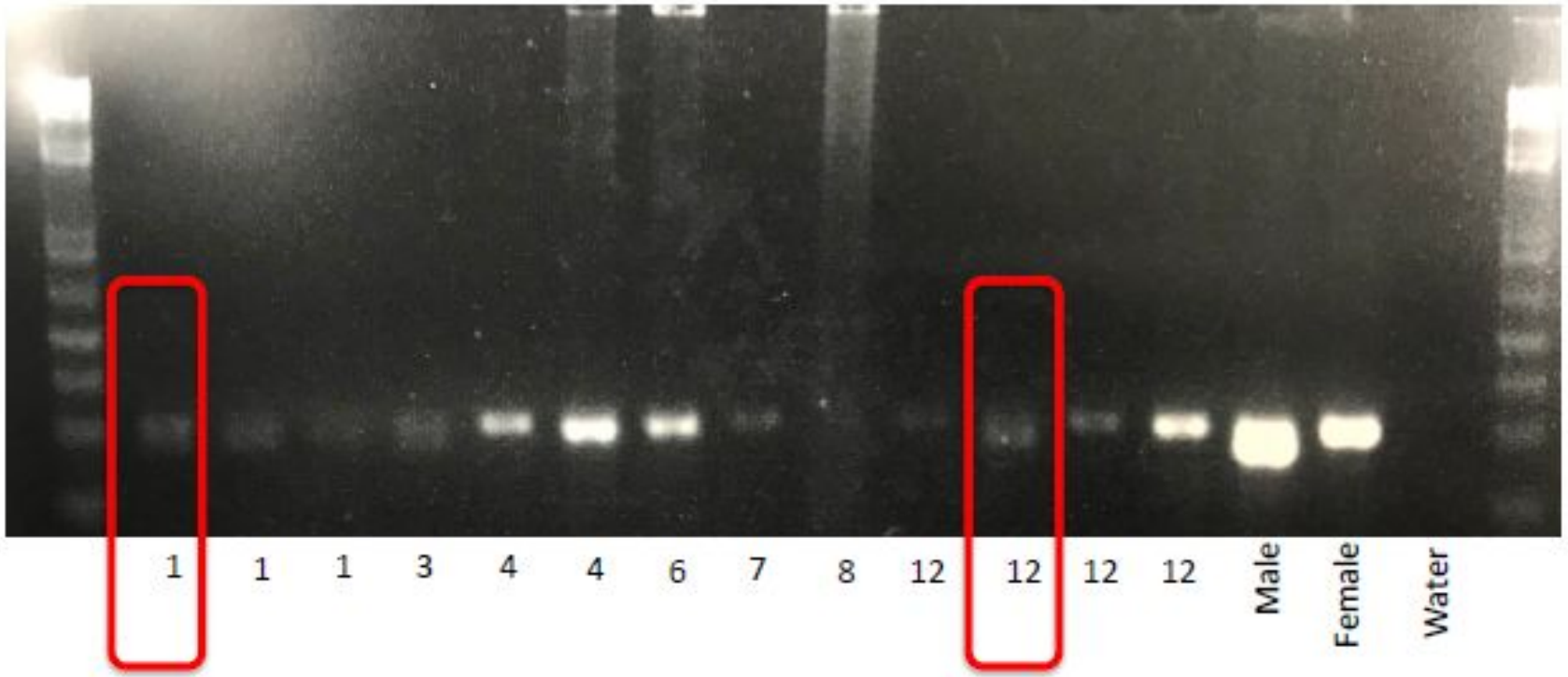
- All colonies appear tw2/+ except 3, which looks like tw2/tw2



iPSC Sexing

Sexing of iPSC colonies

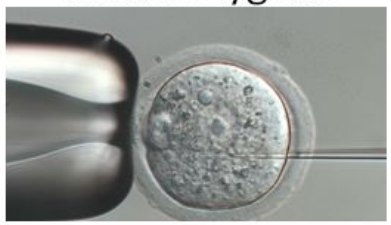
- Repeated sexing PCR
- Colonies 1, 3, and one 12 appear male



Task 3.1.1.3 - Feminizing Y-Shredder - CRISPR mice

Y-Shredding in vivo

Mouse zygote



Centro 41x gRNA
+ Cas9 mRNA

Transfer and harvest at 14 days

XY → XO in vivo!
XO develop ovaries

Assess off-targets
Add to "best" homing system

Adikusuma et al 2017 *Molecular Therapy*

Task 3.1.1.4.1 - Island Selection

PROGRESS

- Regular meetings
- Island searches made and contacts established
- Specific protocols established
- Animal ethics approvals secured/being applied for
- Coordinating collections

Karl Campbell
John Godwin
Toni Piaggio
Aaron Sheils
Margaret Byrne & Keith Morris (AU)
James Russell (NZ

Campbell, K. J., Saah, J. R., Brown, P. R., Godwin, J., Gould, F., Howald, G. R., Piaggio, A., Thomas, P., Tompkins, D. M., Threadgill, D., Delborne, J., Kanavy, D. M., Kuikin, T., Leitschuh, C., Packard, H., Serr, M. & Shiels, A. (submitted) **A potential new tool for the toolbox: Assessing gene drives for eradicating invasive rodent populations**. In: *Island invasives*: IUCN.

Task 3.1.3.2 - Model, Spatial, Stochastic, Individual-Based

Different Gene Drive Strategies and Resistance

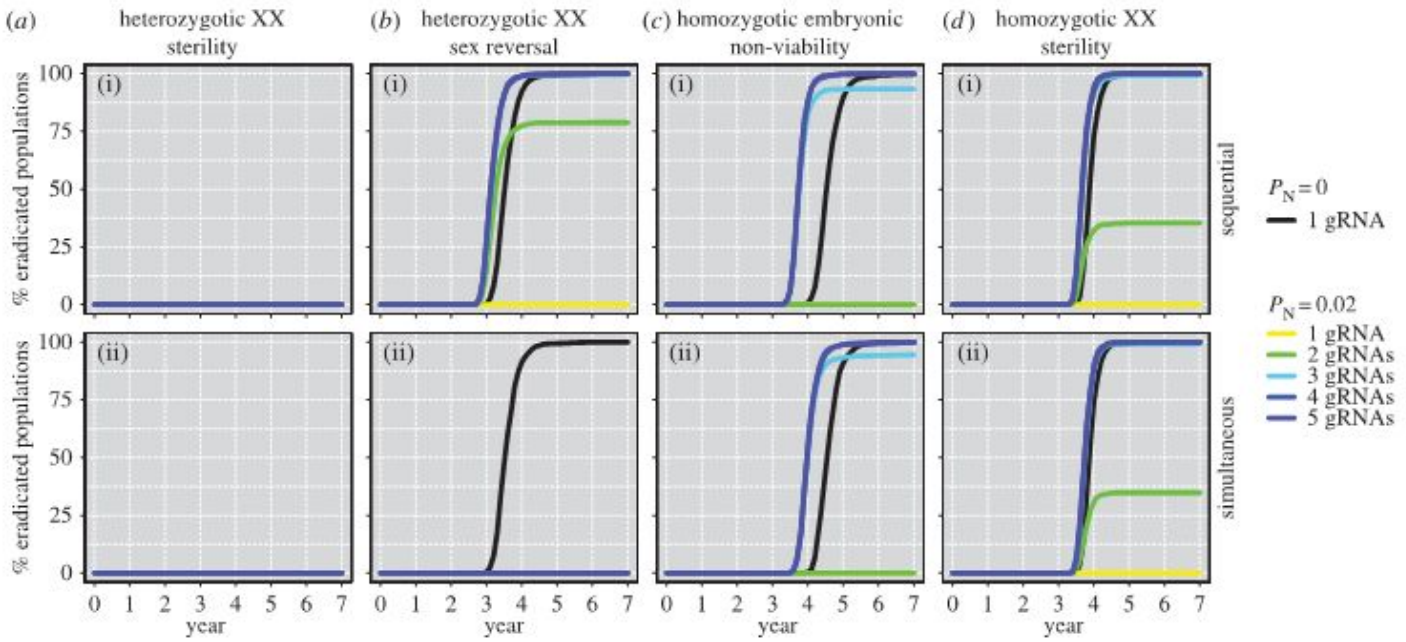


Figure 2. The percentage of eradicated mouse populations over time for different gene-drive strategies, assuming a starting population size of 50 000 individuals and either sequential (independent) or simultaneous gRNA activity on multiple DNA recognition sites. The gene-drive strategies are: (*a*) heterozygotic XX sterility; (*b*) heterozygotic XX sex reversal; (*c*) homozygotic embryonic non-viability; and (*d*) homozygotic XX sterility. Within each strategy, different numbers of multiplexed mRNA guides were tested. Results are shown for simulations that assume the probability of NHEJ occurring following cutting is 0 or 2% (i.e. $P_N = 0$ or 0.02). Note that no simulated eradications occurred for the first gene-drive strategy, so all lines overlap in *a*.

Dodging silver bullets: good CRISPR gene-drive design is critical for eradicating exotic vertebrates

Thomas A. A. Prowse[1], Phillip Cassey[2], Joshua V. Ross[1], Chandran Pfitzner[2], Talia A. Wittmann[2] and Paul Thomas[2,3]

vertebrates. *Proc. R. Soc. B* **284**: 20170799.
http://dx.doi.org/10.1098/rspb.2017.0799

Task 3.1.3.2 - Model, Spatial, Stochastic, Individual-Based

Impact of Resistance on Eradication

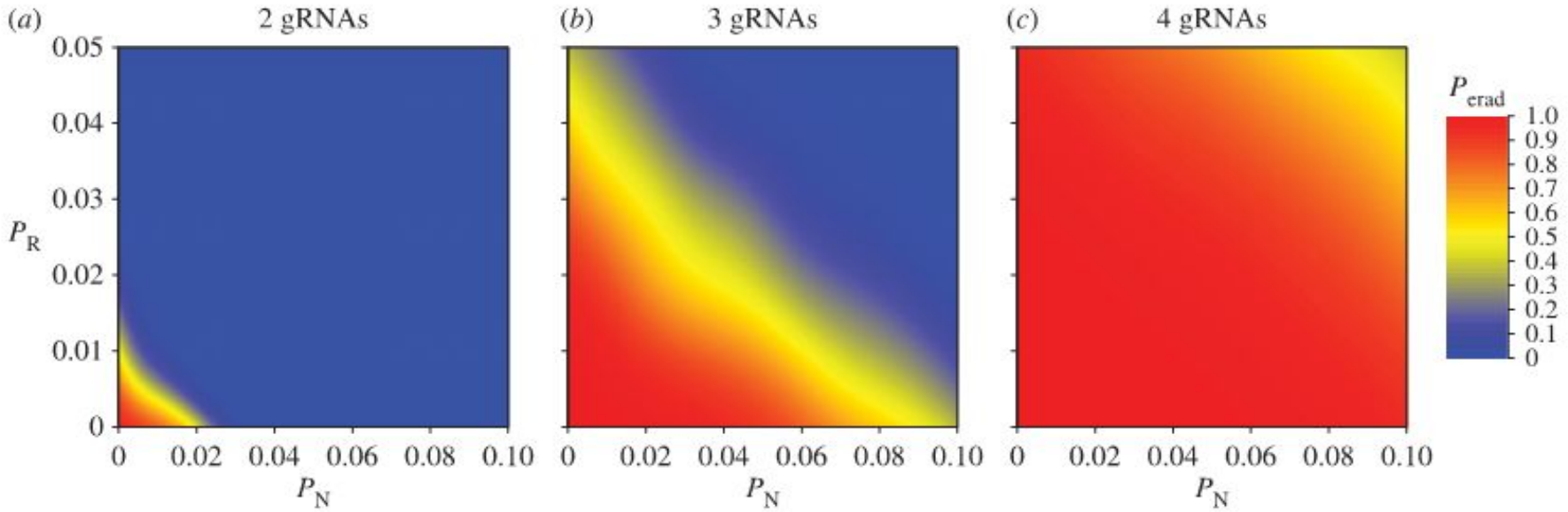
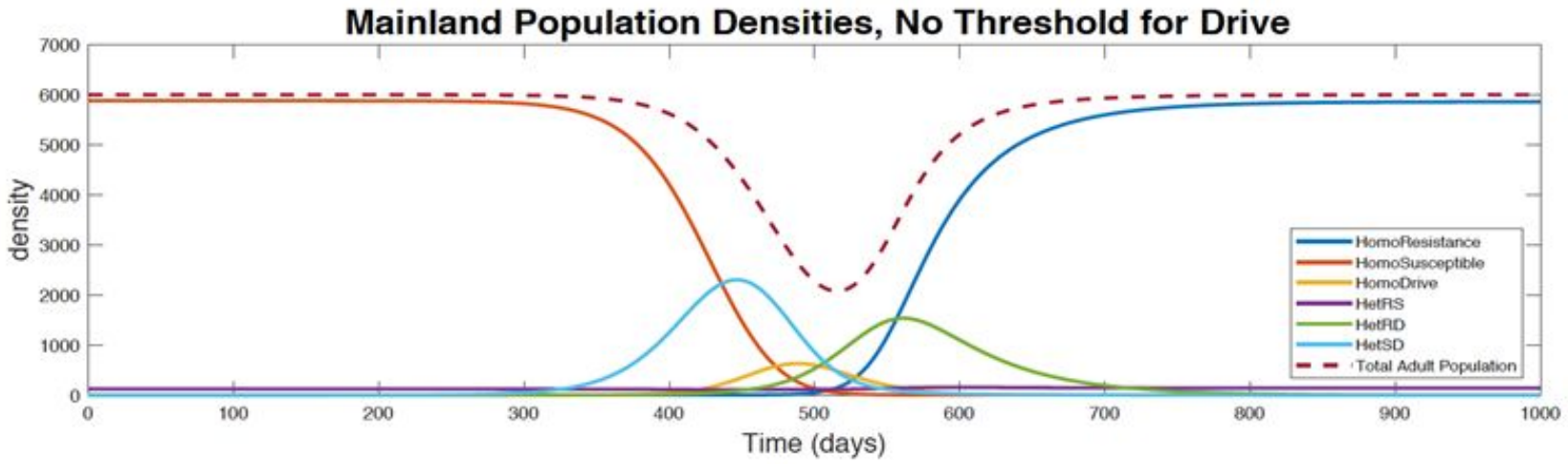


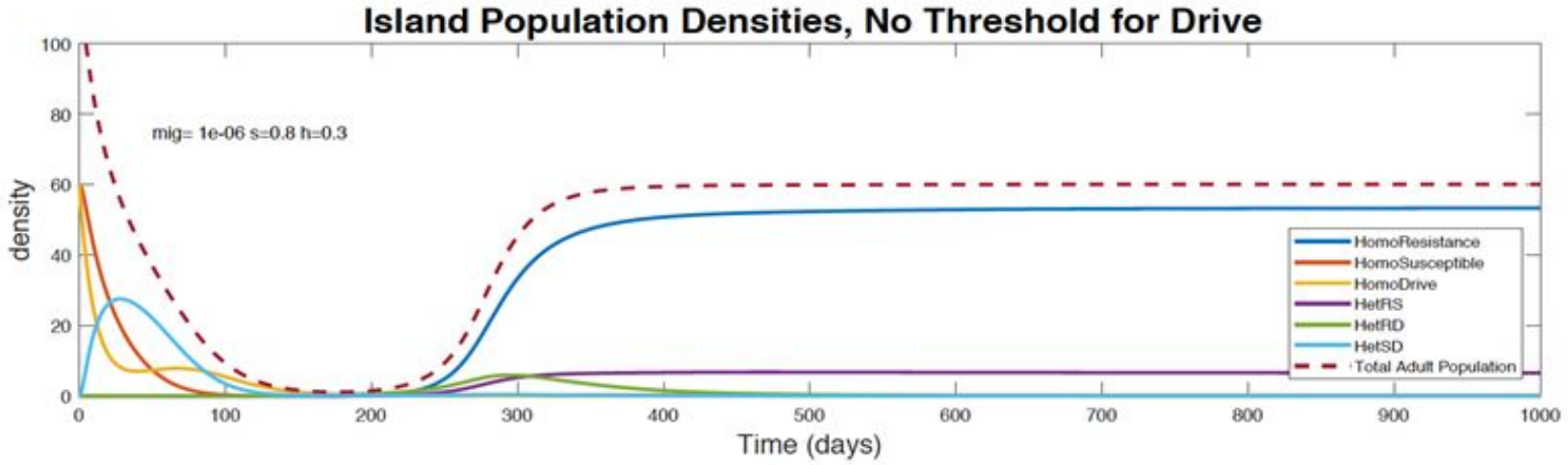
Figure 6. The impact of the probability of NHEJ (P_N) and existing polymorphic resistance (P_R) on the probability of successful mouse eradication (P_{erad}) under the homozygotic XX sterility gene-drive strategy. The results shown assume an island carrying capacity of 50 000 mice, 100 gene-drive carriers used for inoculation, simultaneous gRNA expression and ($a-c$) two, three or four gRNAs. The plotted probabilities are derived from a binomial spatial spline fitted to the sensitivity-analysis output separately for each panel.

Prowse et al. 2017

Task 3.1.3.2 - Model, Spatial, Stochastic, Individual-Based

Locally Fixed Alleles





Task 3.1.4 - US Regulatory Engagement

Guiding Questions

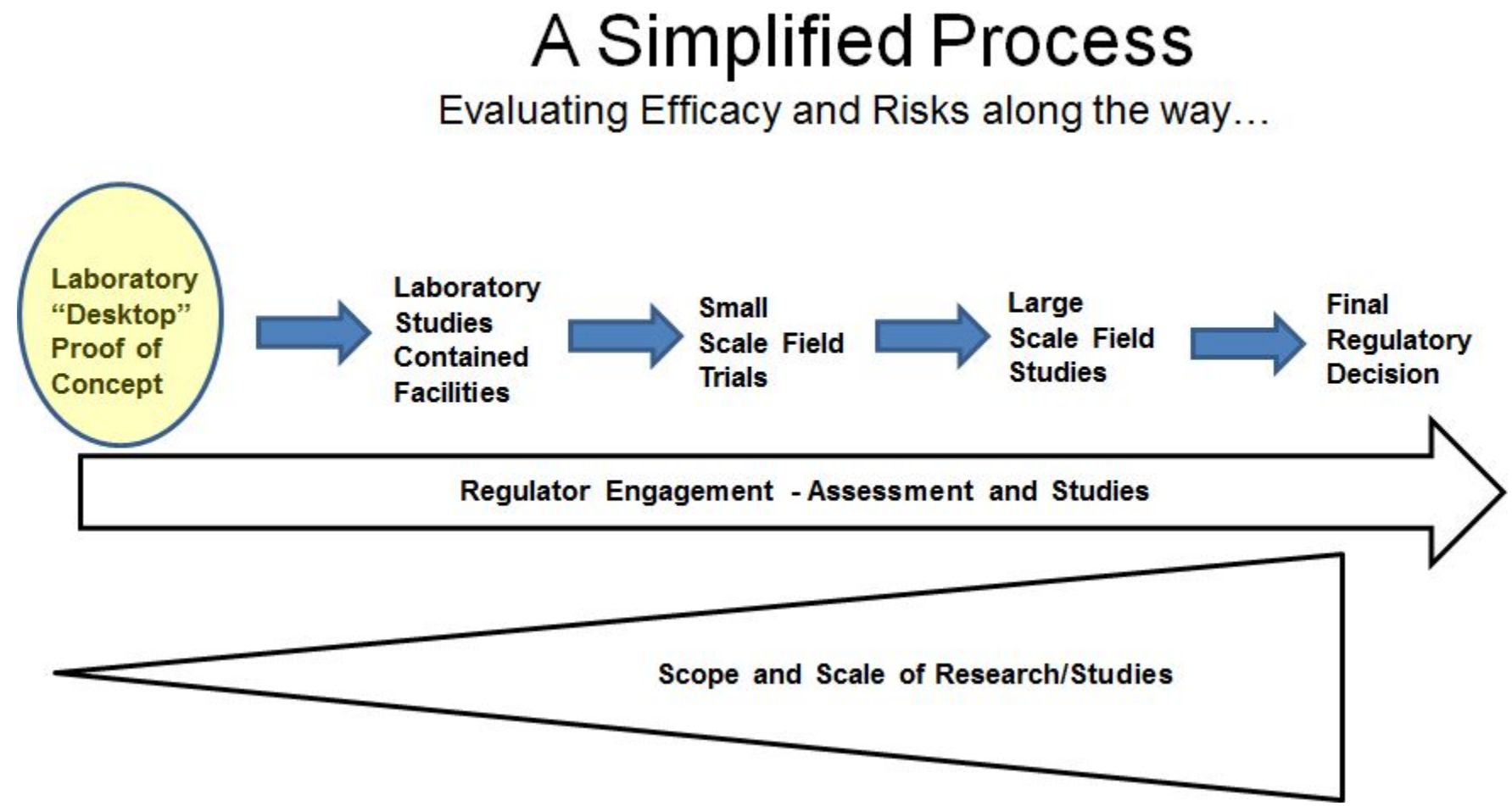
Demonstrate Efficacy – does it work?

Evaluate Safety – Is it Safe? What are the risks?

Risk:Benefit - Does the Benefit Outweigh the risks?

The importance of strict adherence to the regulatory requirements cannot be overstated

Task 3.1.4 - US Regulatory Engagement



What have we been doing?

- ***FDA/EPA/USDA May 2017***: Simultaneous Engagement

- ***Regulatory "Toolkit"*** Discussions August 2017: US NISC initiated

- ***NCSU Safe Genes Meeting Wash. D.C. November 2017***: Invitations for US regulators (EPA, FDA, and USDA) to attend.

- **Review/track regulatory requirements:** General and specific for moving mice to USDA-NWRC

Task 3.1.4 - US Regulatory Engagement

What have we learned?

1. Ambiguity from May getting more clear:
   DARPA LEEDR meeting Friday brought clarity

2. FDA and/or EPA will be the regulatory agency (ESA FWS)

3. Need to identify a sponsor = registrant

4. **Claim and Intent** will assist in determining which of the two agencies.

Proposal for Claim: Reduce a population of House Mouse.

Task 3.1.4 - US Regulatory Engagement

USDA- NWRC Compliance

- APHIS WS NWRC NEPA assessment/requirements
- Animal Welfare Act (IACUC) requirements
- USFWS Interstate transportation
- Health clearance by APHIS CV and CDC

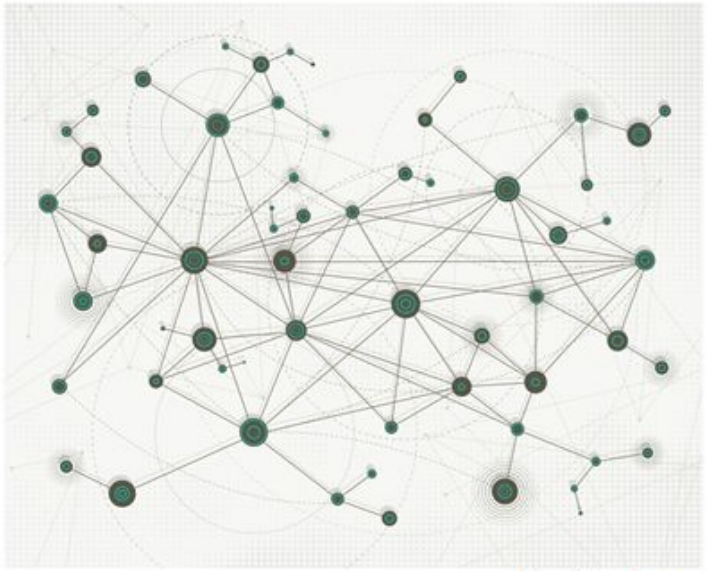
If FDA:

- Establish an INAD file(?)
- FDA study guidance
- GLP/GCP requirements
- Unapproved new animal drug labeling requirements

If EPA:

- EPA study guidance
- GLP requirements
- Unregistered pesticide labeling requirements

3.1.5 - Stakeholder Engagement



blog.taitradio.com

Stakeholder Landscape Analysis
(interests, positions, influences)

M. Farooque (ecastnetwork.org)

Stakeholder Workshop
(scenarios, technical feedback)

Landscape Analysis – Driving Questions

- What is the status of public debate around conventional invasive species eradication on islands?
- What are the mix of interests, and who represents those interests, attending to global, national, regional, and local scales?
- Are there “silent stakeholders” or “dormant stakeholders”?
- What are the design characteristics most valued by stakeholders in terms of a technology/method for eradication?
- What “endpoints” of risk assessment matter most?
- Acknowledging existing opposition to killing any animal, how might the team’s technologies impact that debate (positively and negatively)?
- How might cultural and political diversity impact debates over this technology?

Landscape Analysis – Farallon Islands

- US Fish & Wildlife Service proposed rodent eradication using brodifacoum to eliminate food source that attract owls that later feed on storm petrels.
- Initial EIS, public comment period, revised EIS in 2013.
- Arguments against:
  - Poisoning would cause too much collateral damage (non-target impacts)
  - Eliminating the mice might not be sufficient to protect the storm petrels (lack of confidence in ecological models)
- Next steps
  - Finalize list of key organizations, interest groups, and spokespersons
  - Analyze public comments on EIS

Upcoming Tasks

Anticipated work for next reporting period:

- 3.1.1.1: Continued development of tw2-carrying IPSCs, finalize protocols for genotyping and sexing
- 3.1.1.2: Continued generation/expansion of GM mouse lines for homing analysis.
- 3.1.1.4: permits for obtaining samples collected by Point Blue Conservation Science in Farallons; Animal ethics approval in Western Australia
- 3.1.3: In person meeting with NCSU and Univ of Adelaide Modelers
- 3.1.4: Receive clarity regarding primary jurisdiction of technology regulation
- 3.1.5: Continue process of identifying stakeholders for landscape analysis and finalize development of 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

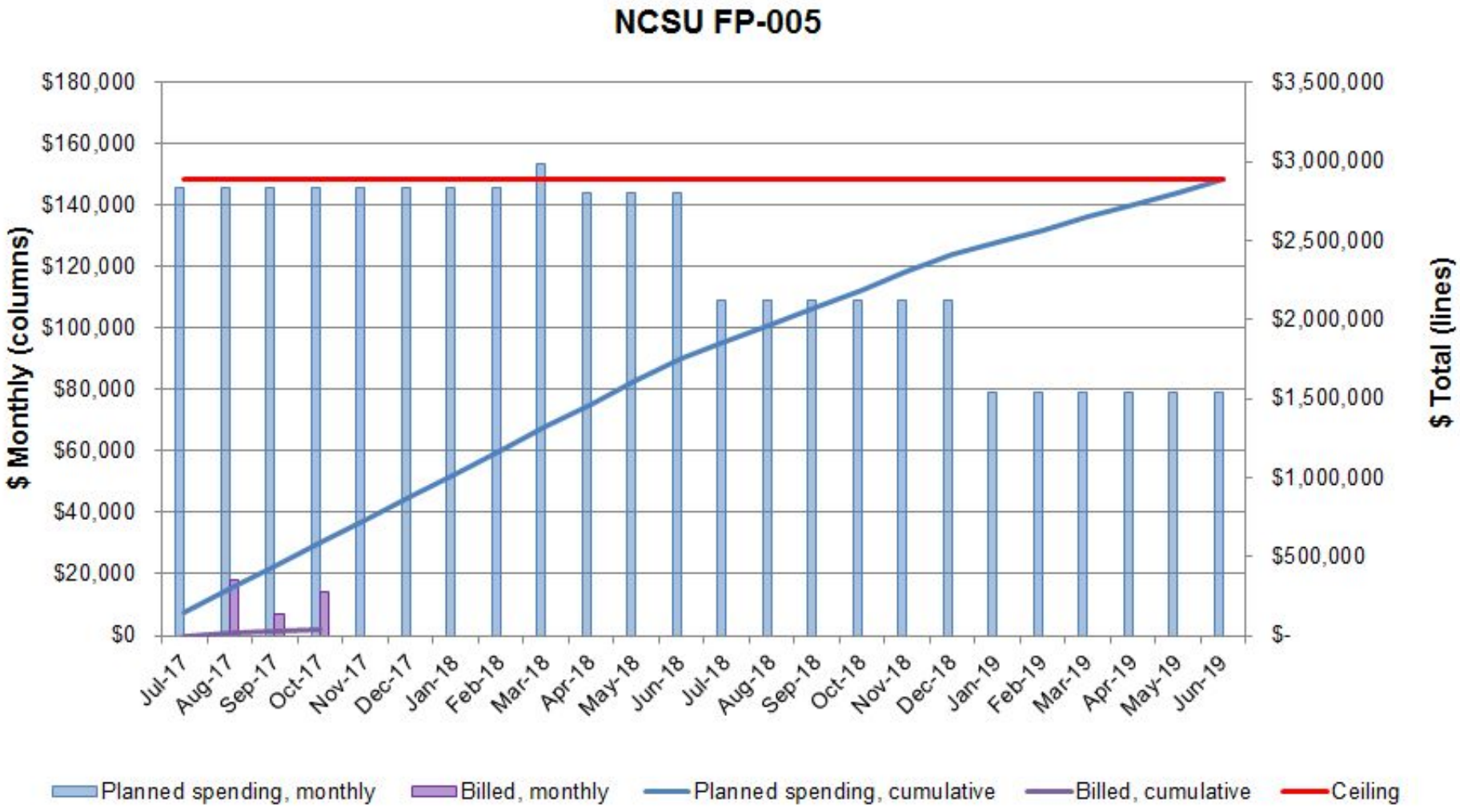
SOW Task #		Contract Start	Due Date	Actual Start	Predicted Finish	Status (%)	Exit Criteria (Milestones and Deliverables)	Reason for Delay
3.1.1.1	Engineer t-Sry mice	5/1/2017	2/1/2019	6/1/2017	In progress	17%	Engineer t-Sry mice to express Sry under doxycycline control	Awaiting ACURO approval
3.1.1.2	Generation 1 drive mice	5/1/2017	11/30/18	7/1/2017	In progress	17%	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	12%	Identify population-specific Private Alleles in six mouse island population and adjacent mainland populations	Island selection process is on track
3.1.3	Mathematical modeling of performance of Genome editors	5/1/2017	2/28/19	6/30/2014	2/28/19	13%	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	16%	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	12%	Draft technology scenarios, Workshop report with recommendations, stakeholder map	N/A

Public Affairs and Public Engagement

- Publications
  -
- Meetings
  - Planned in person meeting with NCSU and Univ of Adelaide modelers in Raleigh in early December
- Items for Public Release
  -

Additional slides to consider as needed…

Detailed spend plan - as of Nov 10, 2017



Spend Plan Deviation Details/Mitigation plan

- NCSU and all subs are working together to ensure all required administrative forms and actions are completed.
- This should be fully completed by end of year 2017.