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: 5/1/2017 to 8/8/2017

Briefing Prepared for Renee Wegrzyn

August 10, 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):

- None to report for DARPA project period

Challenges: ACURO approval process period

Technical Progress - Executive Overview

Technical progress update:

- Editing of t-haplotype – four gRNAs identified that are effective for cutting *in vitro*. Currently testing cutting efficiency in mouse embryonic fibroblasts carrying t-haplotype and reprogramming fibroblasts into pluripotent stem cells.
- Generation 1 synthetic drive mouse development
  - (3.1.1.2) fast tracking preparation of Generation I drive mice through SA Genome Editing. Reagents repair templates for "gRNA Cas9" and "target" mice are synthesised and tryosinase gRNA prepared and validated (awaiting ACURO approval to make mice).
  - 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.
- Identification of population-specific, locally-fixed alleles ('Private alleles')
  - Established collaboration with Department of Environment in Western Australia to identify suitable islands for sampling in that region
  - Shiels (USDA-NWRC) coordinating U.S. Islands list for sampling
  - Godwin lab ACURO protocol for collections approved
- Regulatory Engagement
  - New Zealand: In-depth roundtable discussion with representatives of twelve agencies
  - U.S.: Three discussions, first with FDA and then a combined meeting with FDA, USDA, and EPA representatives; Currently waiting on these agencies for assessment of regulatory authority responsibility
- Stakeholder Engagement: Landscape Analysis planning is underway.

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	10%	Engineer t-Sry mice to express Sry under doxycycline control	Testing editing approach in cell system
3.1.1.2	Generation 1 drive mice	5/1/2017	11/30/18	7/1/2017	In progress	5%	Assess stability, efficiency of CAS9-mediated germline and zygotic homing	Optimizing reagents for mouse generation (ACURO approval pending)
3.1.1.3	Feminizing Y-shredder drive	5/1/2017	2/28/19	1/1/2018	In progress	10%	Develop an efficient feminizing endonuclease gene drive (Y-shredder)	Effective Y-shredding achieved *in vitro*
3.1.1.4	Identify Population-specific alleles	5/1/2017	2/28/19	6/30/2017	In progress	5%	Identify population-specific Private Alleles in six mouse island population and adjacent mainland populations	Island selection in progress; Contract pending with USDA-NWRC
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	5%	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	5%	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	Not yet started	In progress	5%	Draft technology scenarios, Workshop report with recommendations, stakeholder map	Landscape analysis planning is underway

Upcoming Tasks

Anticipated work for next reporting period:

- 3.1.1.1: Testing gRNAs for *sry* insertions in t-allele in mouse embryonic fibroblasts, reprogramming t-allele carrier fibroblasts into pluripotent stem cells
- 3.1.1.2: Continued optimization of reagents for drives, beginning of mouse work when ACURO approval is official
- 3.1.1.4: Narrow candidate island list and initiate approvals process for collections
- 3.1.3: Building on approaches developed in Proc. Royal Society paper (Prowse et al., 2017) and initial development of a two-patch model to assess locally-fixed allele approach
- 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: 8/8/2017

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	10%	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	5%	Generation of 6 transgenic lines for Generation 1 homing experiments	Awaiting ACURO approval. 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	5%	Identify population-specific Private Alleles in six mouse island population and adjacent mainland populations	Island selection process is on track, but contract is still pending with USDA-NWRC
3.1.3	Mathematical modeling of performance of Genome editors	5/1/2017	2/28/19	6/30/2014	2/28/19	5%	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	5%	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	MM/DD/YYYY	2/28/2019	5%	Draft technology scenarios, Workshop report with recommendations, stakeholder map	N/A

Public Affairs and Public Engagement

- Publications
  - None from Safe Genes project specifically to report yet
  - Peer-reviewed publications - relevant to project, but preceded Safe Genes : Prowse et al. (2017) *Dodging silver bullets: good CRISPR gene-drive design is critical for eradicating exotic vertebrates.* Proc. Royal Society B, published 8/8/2017
  - Popular Press articles – relevant to project, but preceded Safe Genes
    - http://www.audubon.org/magazine/summer-2017/how-genetically-modified-mice-could-one-day-save
    - http://e360.yale.edu/features/should-new-genetic-engineering-be-used-as-a-conservation-tool
- Meetings
  - Island Invasives Conference (Dundee, Scotland) – two talks related to overall project, but developed from efforts preceding Safe Genes project
  - Strong support and interest from invasive species management practitioner community at conference
- Items for Public Release
  - None as yet

Compliance

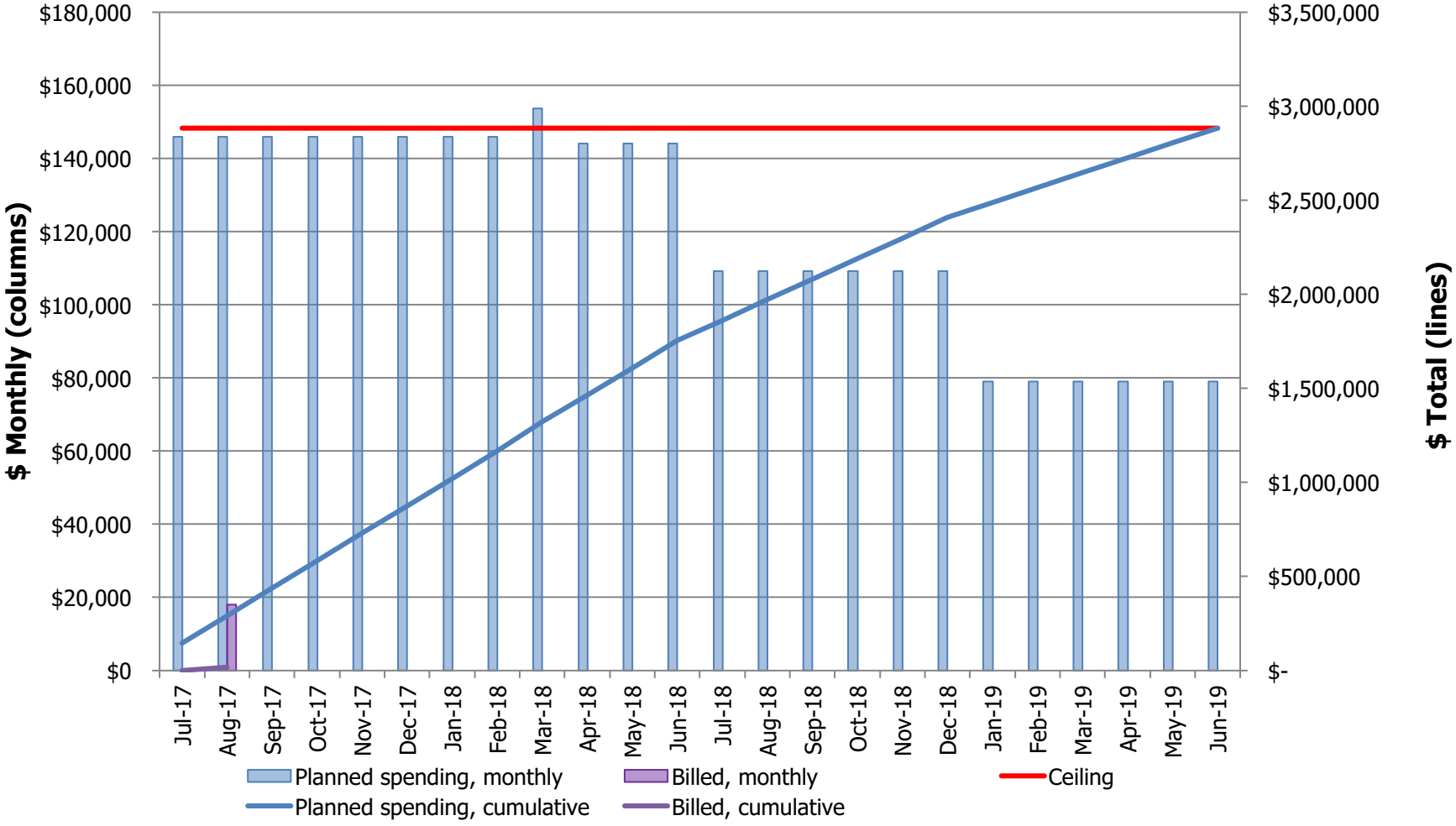
- Animal protocols
  - Campus Animal care and use committees: Approved for NCSU, University of Adelaide, Texas A&M
  - ACURO: Approved for NCSU, pending
- HSR: Not applicable and IRB review not required, documentation being prepared

Additional Items for Discussion

- *Administrative*
  - *Milestone timing – Contract executed 7/6/2017*

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