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: 9/14/2017 to 10/10/2017

Briefing Prepared for Renee Wegrzyn

October 12, 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):

- Regulatory
- 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

Challenges: ACURO approval process period

Technical Progress - Executive Overview

Technical progress update:

- Engineering of t-Sry mice (3.1.1.1)
    - IPSCs are in third passage, protocol development for genotyping and sexing IPSC colonies
- Generation 1 synthetic drive mouse development (3.1.1.2)
    - "gRNA Cas9" founder mouse generated and validated.
- Identification of population-specific, locally-fixed alleles ('Private alleles') (3.1.1.4)
    - Initial list of 11 potential islands developed that are good fit for criteria:
        - 5 in US territory - continuing challenges with advancing efforts for three USVI islands due to effects of Hurricanes Irma and Maria
        - 6 in Australia - Determining best islands from project perspective and approach to collection.
    - Continuing work on US permitting process to bring in samples
        - Hiring Post-doc at NCSU with wild rodent sampling and tracking experience (11/1/17)

Technical Progress - Executive Overview

Technical progress update:

Modeling (3.1.3)

- No updates from September report

- Regulatory Engagement (3.1.4)
  - Discussions with EPA and USDA regulators (separately)

•Stakeholder Engagement (3.1.5): Landscape Analysis

- Research conducted on media coverage and grey literature on rodent eradications and gene drives to produce initial list of potential stakeholders to interview (sub-set to be invited to stakeholder workshop in fall 2018)
- Graduate student at NCSU (not funded by DARPA) completed a dissertation chapter analyzing media coverage of rodent eradications (submitted to Conservation Bio).
- Engagement team begins using Zotero for collaborative research and collection of data to support landscape analysis.

Milestones and Task Status Overview

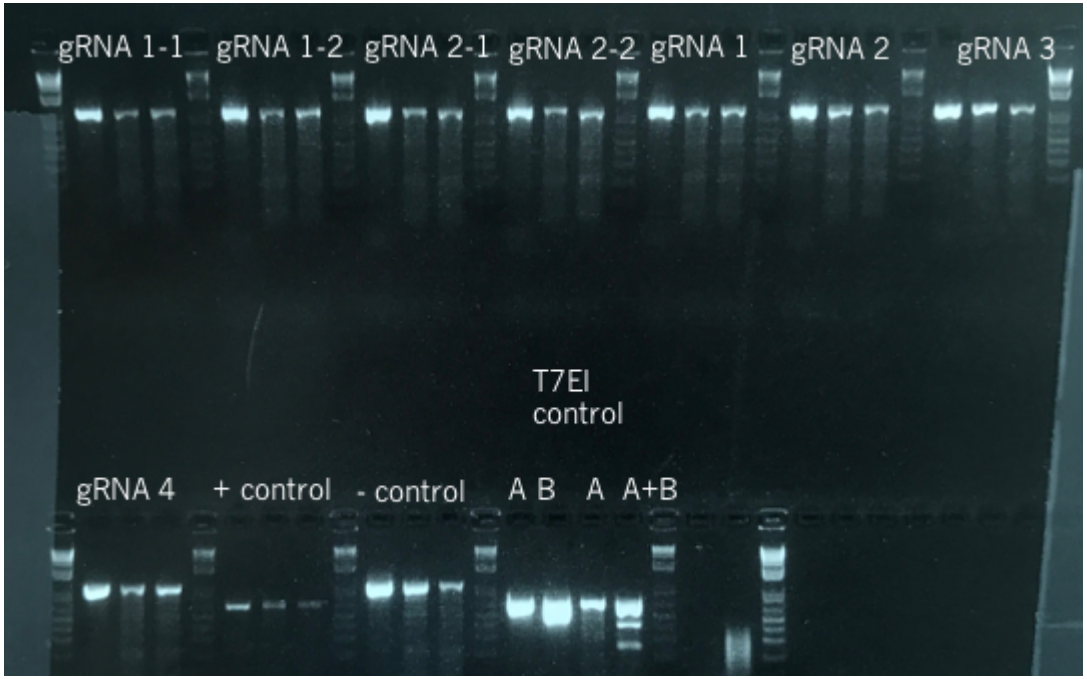
Restoring Ecosystems and Biodiversity through Development of Safe and Effective Gene Drives

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, continue developing IPSCs
3.1.1.2	Generation 1 drive mice	5/1/2017	11/30/18	7/1/2017	In progress	15%	Assess stability, efficiency of CAS9-mediated germline and zygotic homing	ACURO approval obtained "Target" founder mice generated CMV-Cas9 mice imported and validated. "gRNA (CAS9)" mouse generated
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	Hurricane effects in USVI, Working with Dept Parks and Wildlife in Western Australia
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	12%	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



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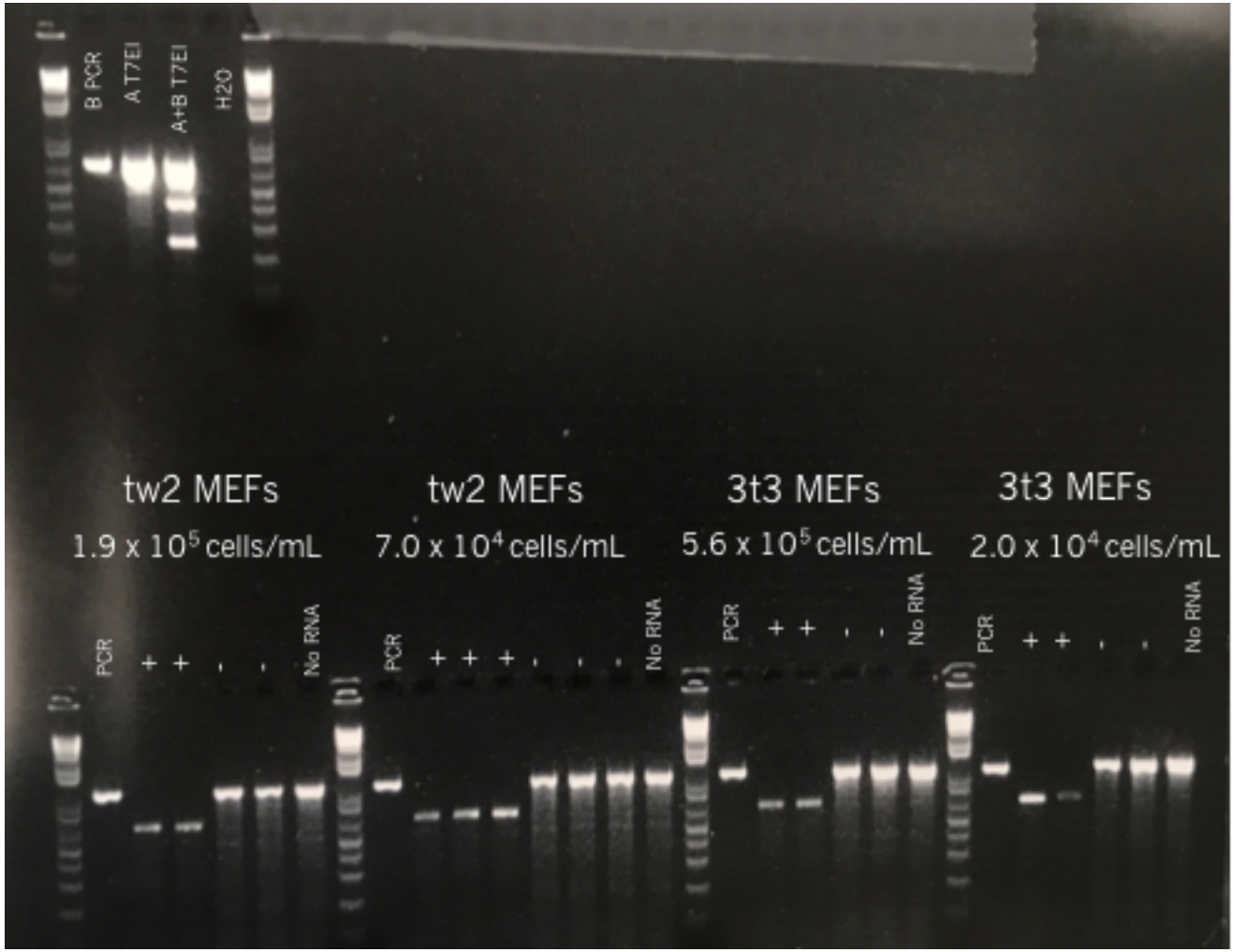
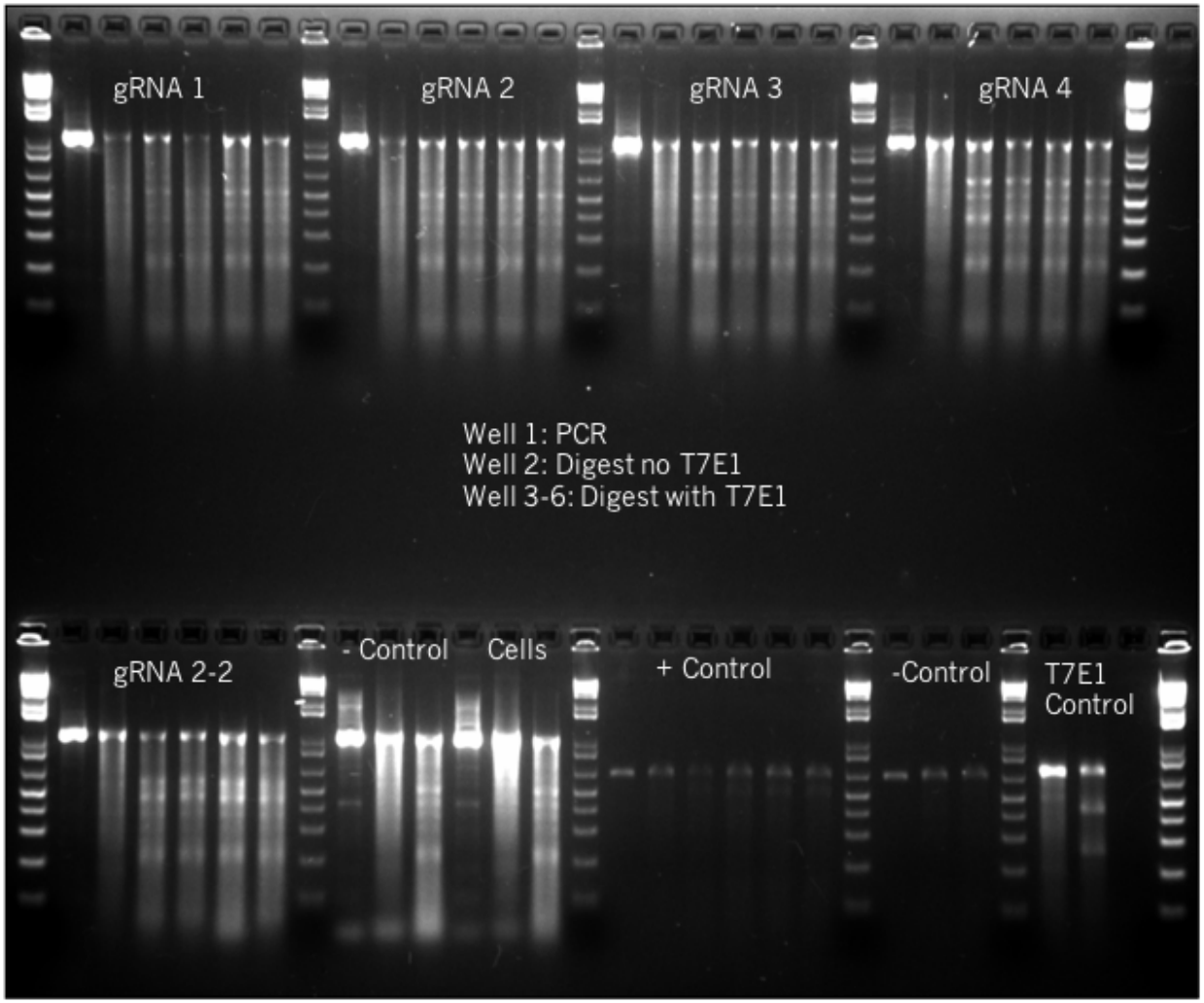


Task 3.1.1.1 - Engineer t-Sry mice



3.1.1.2 Generation I Drive mice (Target and CMV-CAS9)

- Transmission of “Target” allele confirmed from breeding of founder mice
- CMV-CAS9 colony established

Upcoming Tasks

Anticipated work for next reporting period:

- 3.1.1.1: Continued development of tw2-carrying IPSCs, refine protocols for genotyping and sexing
- 3.1.1.2: Generation/expansion of GM mouse lines for homing analysis.
- 3.1.1.4: Narrow candidate island list; permits, preparations, approvals for collections in Farallons
- 3.1.3: Continue development of two-patch model to assess locally-fixed allele approach

  •3.1.4: Conduct workshop for US regulatory system for Gene Drive Development for Invasive Species management application; Participate in LEEDR workshop involving EPA, FDA representatives
- 3.1.5: Continue process of identifying stakeholders for landscape analysis and development of protocol for outreach to stakeholders; Related: Participate in *'Talking about Gene Drives'* workshop in Baltimore, MD

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	15%	Generation of 6 transgenic lines for Generation 1 homing experiments	Delay in contract between UA and NCSU (executed Oct 2017).
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, continuing 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	12%	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	10%	Draft technology scenarios, Workshop report with recommendations, stakeholder map	N/A

Public Affairs and Public Engagement

- Publications
  - None in this reporting period
- Meetings
  - Commission on Genetic Modification, Rotterdam, Netherlands, 10/19-20 (Godwin invited and at no cost)
  - Safe Genes LEEDR (DARPA, 11/3)
  - Talking about Gene Drives (FNIH, 11/4)
  - GBIRd and Safe Genes, 11/5-6
- Public Engagement/Outreach
  - 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

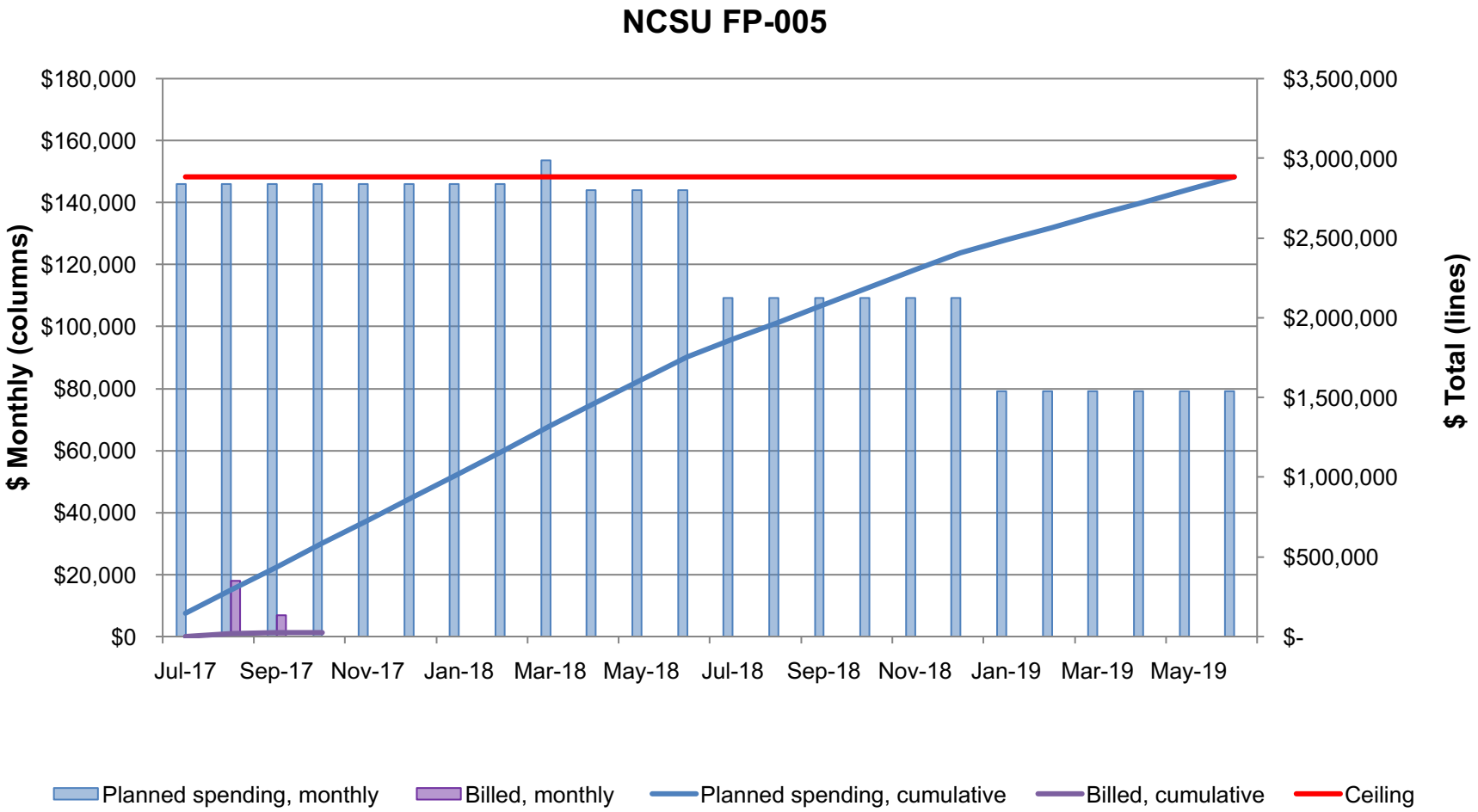
- Animal Use protocols

Additional Items for Discussion

- Upcoming GBIRd and Safe Genes team meeting on 11/5-6

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