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: 11/06/2017 to 12/10/2017

Briefing Prepared for Renee Wegrzyn

December 14, 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 - 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

Challenges:

- ACURO approval process period
- Hurricanes in Caribbean necessitating alternate island selections

Technical Progress - Executive Overview

Technical progress update:

- Engineering of t-Sry mice (3.1.1.1)
  - Initial set of iPSCs had to be discarded, but new iPSC set derived from male t-allele carrier developed and growing well
- Generation 1 synthetic drive mouse development (3.1.1.2)
  - Validation of Cas9 expression in the testis of Vasa-Cas9 transgenic line
  - Mosaic mice generated from CAS9 zygotic homing experiments
- Identification of population-specific, locally-fixed alleles ('Private alleles') (3.1.1.4)
  - US Islands chosen and sampling partly completed
  - Australian Islands: candidate islands identified, collaboration established for sample with Dept Parks and Wildlife (State of Western Australia)

Technical Progress - Executive Overview

Technical progress update:

- Modeling (3.1.3)
  - Incorporation of social structure/mating patterns into modeling of gene drive function initiated

- Regulatory Engagement (3.1.4)
  - Regulatory - FDA will be initial US regulatory agency with authority over this project

- Stakeholder Engagement (3.1.5): Landscape Analysis
  - Refinement of stakeholder list for interviews and interview protocol
  - Landscape analysis initiated

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	20%	Engineer t-Sry mice to express Sry under doxycycline control	Initial iPSCs discarded, new male t-allele iPSCs generated, growing
3.1.1.2	Generation 1 drive mice	5/1/2017	11/30/18	7/1/2017	In progress	20%	Assess stability, efficiency of CAS9-mediated germline and zygotic homing	CMV-Cas9 drive testing undderway Vasa-Cas9 expression validated (qPCR) Vasa-Cpf1 founders 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	15%	Identify population-specific Private Alleles in six mouse island population and adjacent mainland populations and conduct populaion genetic analyses	Samples have been collected rfom Farallon Islands and Midway. Job listing for post-doc to do library prep and analyses to be listed Dec, 2017
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	15%	Spatial, stochastic individual-based model for mouse population and analysis of gene drive strategies	Incorporation of social structure/mating patterns into modeling of gene drive function initiated
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	15%	Draft technology scenarios, Workshop report with recommendations, stakeholder map	Stakeholder engagement and Landscape analysis initiated

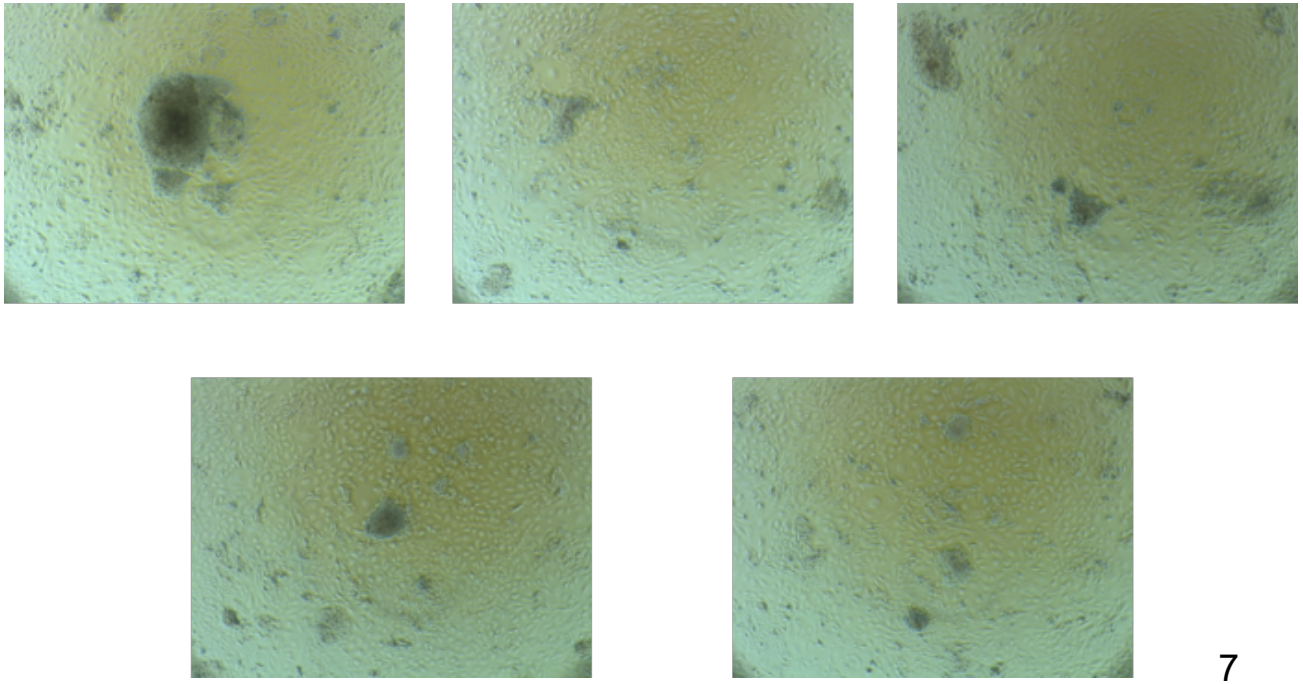
Task 3.1.1.1 - Engineer t-Sry mice

•Setback: Initial set of iPS cells became contaminated and had to be discarded

• New iPS cells: MEFs (mouse embryonic fibroblasts made from a male tw2/+ were transduced

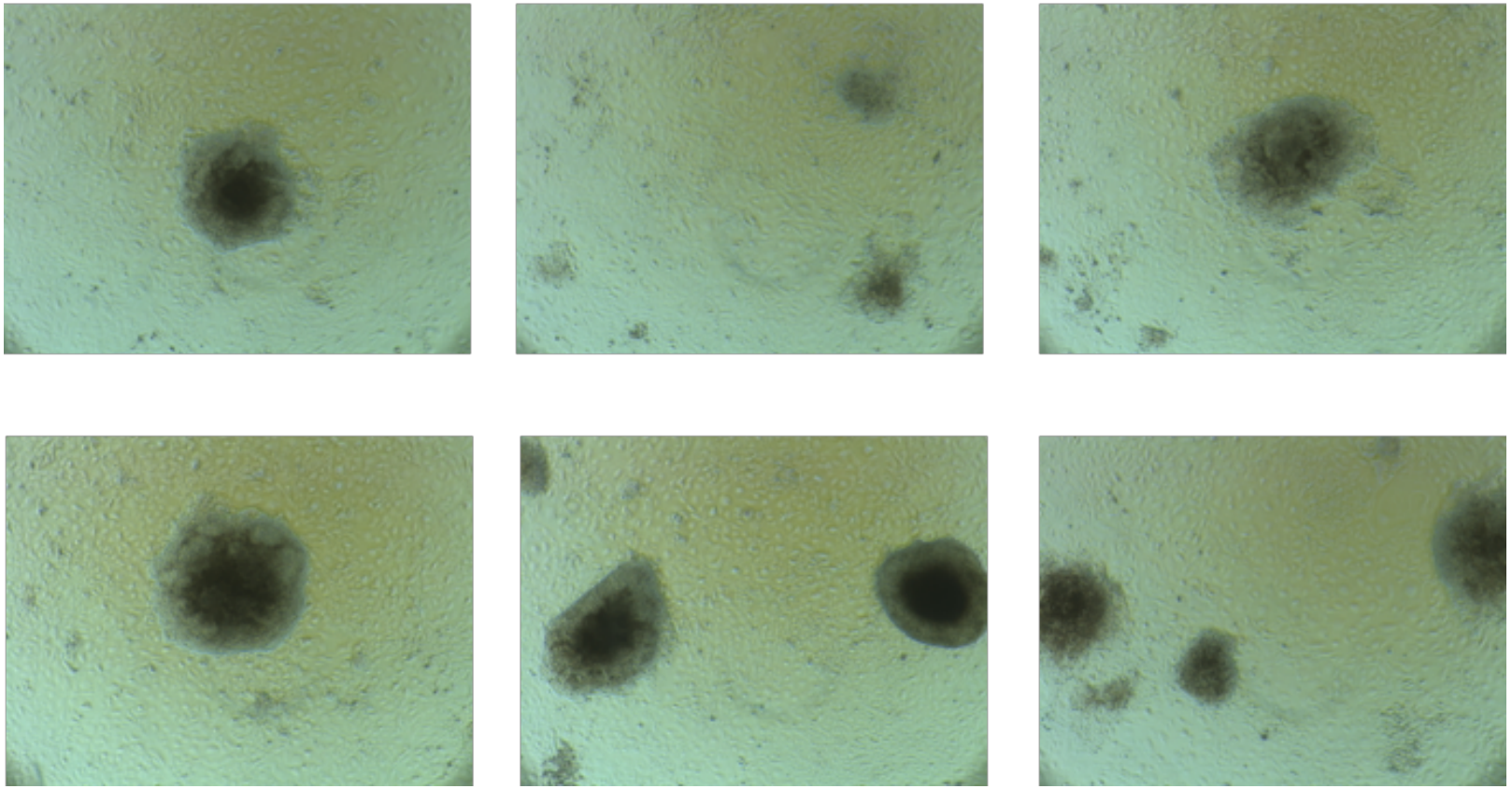
- Advantage: sexing and genotyping of cells not necessary
- Now passaged for a second time
- Growth is more rapid than the initial set of iPS cells

Day 20



Task 3.1.1.1 - Engineer t-Sry mice

- Day 30



Task 3.1.1.4 - Locally-fixed alleles

- Identification of population-specific, locally-fixed alleles
- US Islands chosen:
  - Southeast Farallon Island (as noted in SOW), 130 mouse samples collected in November 2017
  - Sand Island, Midway Atoll, 50 mouse samples obtained thus far
    - Collaboration established to obtain mouse samples from Honolulu Airport area (point of origin and return from Midway Atoll)
- Australian Islands:
  - Candidate islands identified
  - Collaboration established for sample with Dept Parks and Wildlife (State of Western Australia),
    - animal ethics approval considered on 12/6/17, more information requested (approval expected by 12/15)
- Post-doc hired at NCSU with wild rodent sampling, tracking, transport experience
- Seeking to hire postdoc to undertake genomic studies including library preparation and data analysis

Task 3.1.3 - Modeling

- Appointment of modeling postdoc ([redacted], U. of Adelaide)
- Meeting between NC State and U. of Adelaide modeling groups (NCSU, 12/7-8)
  - Discussions of models needed for the SNE and/or outdoor enclosure (if pursued)
- Work (U. of Adelaide) on impact of mating structure and demographics on success of gene drives --- will inform both SNE model and more detailed realistic ecological models
- Safe Genes related: acceptance of paper on spatially self-limited gene drives (comparison of Daisy Drive and Engineered Underdominance) [Dhole, Vella, Lloyd & Gould; Evolutionary Applications https://doi.org/10.1101/159855]

Task 3.1.4.1 - Regulatory Engagement

- EPA and FDA engaged and exposed to technical research progress, November 2017.
- Regulatory stepwise pathway identified for laboratory testing of mouse construct.
- FDA confirmed as primary regulating agency, and conversation on process initiated by email communication.

Task 3.1.5 - Stakeholder Engagement

- Stakeholder Engagement (3.1.5): Landscape Analysis
  - Refinement of stakeholder interview protocol with GBIRd team input
  - Refinement of list of stakeholders to interview
  - Collection of selected media coverage on gene drives to inform landscape analysis

Upcoming Tasks

Anticipated work for next reporting period:

- 3.1.1.1: Continued development of male tw2-carrying IPSCs
- 3.1.1.2: Generation/expansion of GM mouse lines for homing analysis.
- 3.1.1.4: Initiate genetic analyses for Farallons, Midway samples; move towards obtaining Australian samples
- 3.1.3: Continue development of two-patch model to assess locally-fixed allele approach and incorporation of social structure/mating patterns into modeling of gene drive function
- 3.1.4: Consultation with US regulators
- 3.1.5: Continue refinement of stakeholder list for interviews in landscape analysis

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	20%	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	20%	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	15%	Identify population-specific Private Alleles in six mouse island population and adjacent mainland populations	Post-doc hiring in progress
3.1.3	Mathematical modeling of performance of Genome editors	5/1/2017	2/28/19	6/30/2014	2/28/19	15%	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	15%	Draft technology scenarios, Workshop report with recommendations, stakeholder map	N/A

Public Affairs and Public Engagement

- Publications
  - Safe Genes related: acceptance of paper on spatially self-limited gene drives (comparison of Daisy Drive and Engineered Underdominance) [Dhole, Vella, Lloyd & Gould; Evolutionary Applications https://doi.org/10.1101/159855]
- Meetings
  - Commission on Genetic Modification, Rotterdam, Netherlands, 10/19-20/17 (Godwin invited, no cost to *Safe Genes* project)
  - Safe Genes LEEDR, Arlington, VA (DARPA, 11/3/17)
  - *Talking about Gene Drives* workshop, Baltimore, MD (FNIH, 11/4/17)
  - Annual program meeting, Washington, DC (11/5-6/17)
  - New York Invasive Species Research Institute conference, Ithaca, NY (11/13-14/17) (Godwin invited, no cost to *Safe Genes* project)
  - Funding obtained from Australian Academy of Science for Gene Drive meeting in 2018 (Fenner Conference, no cost to *Safe Genes* project)
- Public Engagement/Outreach
  - Press outreach in response to Esvelt publications (quotes in Quantas, Gizmodo, NYT); submission of NYT editorial (not published)
- Items for Public Release
  - None as yet

Compliance

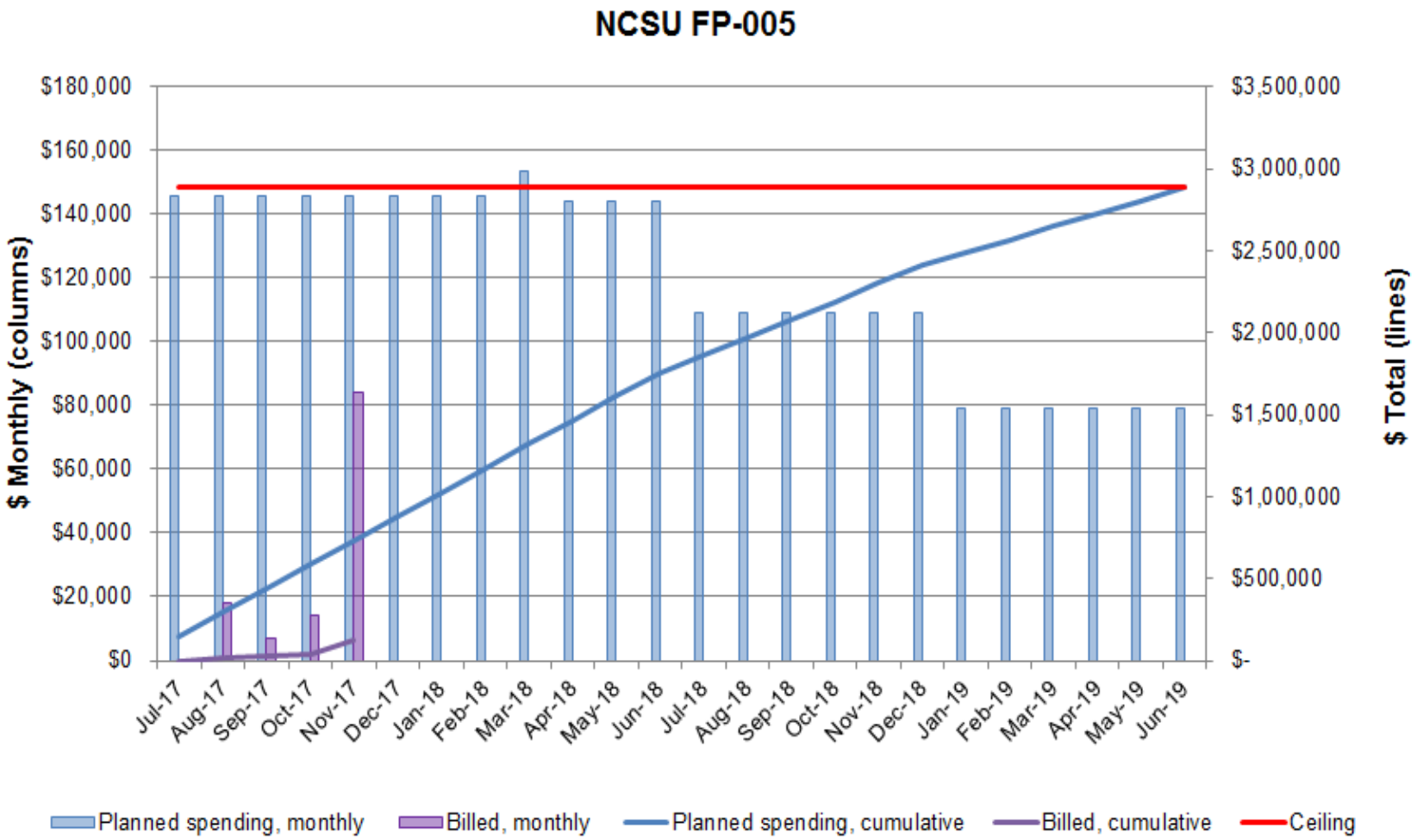
- Animal Use protocols
  - Acuro approval pending for Texas A&M component of project.

Additional Items for Discussion

- Clarification from program about distinguishing Safe Genes-specific activities
- Questions about possible rebudgeting for support of Australian collections of mice
- Update on potential enclosure studies of non-transgenic mice to examine population effects of sex ratio biasing.
- Format for Monthly Technical Update

Detailed spend plan

Financials



Spend Plan Deviation Details/Mitigation plan