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: [01/17/18-2/6/2018]

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 (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
- Vasa-Cas9, Vasa-Cpf1 and CMV-Cas9 transgenic mice generated

Challenges: ACURO approval process period, new guidance on procedures

Technical Progress - Executive Overview

Technical progress update:

- Engineering of t-Sry mice (3.1.1.1):
  - Have now derived embryonic stem cells (ES) and induced pluripotent stem (iPS) cells from t-complex mice
  - Have received ACURO approval for studies
- Generation 1 CRISPR Drive (3.1.1.2):
  - Results indicate that zygotic homing is unlikely to be a viable strategy
- Y-shredder (3.1.1.3):
  - Lead candidate for Y-shredder mouse identified, pending off-target screening in ES cells
- Island selection and sampling (3.1.1.4):
  - Island and mainland samples mostly gathered for one island, half finished for another , and progress on Australian collections
- Modeling (3.1.3): Refinement and write-up of modeling of locally fixed allele impacts on gene drive function on island and mainland populations.
- Regulatory Engagement (3.1.4): No significant progress to report
- Stakeholder Engagement (3.1.5):
  - Pilot interviews complete, protocol finalized, and prioritized stakeholder list for interviews complete

Milestones and Task Status Overview

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

Active Task Status – Past Month

NCSU Safe Genes: Active Task Status - :Dec 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	26%		Engineer t-Sry mice to express Sry under doxycycline control	ES and iPS cells derived from t-complex mice
3.1.1.2	Generation 1 drive mice	5/1/2017	11/30/18	7/1/2017	In progress	25%		Assess stability, efficiency of CAS9 & CPF1-mediated germline and zygotic homing	Zygotic homing not apparent. Generating large deletions in mosaic patchy mice.
3.1.1.3	Feminizing Y-shredder drive	5/1/2017	2/28/19	1/1/2018	In progress	20%		Develop an efficient feminizing endonuclease gene drive (Y-shredder)	Optimising PCR analysis for off-target screening.
3.1.1.4	Identify Population-specific alleles	5/1/2017	2/28/19	6/30/2017	In progress	24%		Identify population-specific Private Alleles in six mouse island population and adjacent mainland populations and conduct population genetic analyses	Post-doc recruitment advanced Progress on samples from US sites and organizing Australian tissue collection.
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		1%		Description of Adverse Outcome Pathways	Commenced, start delayed by contractual delays.
3.1.3	Mathematical modeling of performance of Genome editors	5/1/2017	2/28/19	6/30/17	In progress	25%		Spatial, stochastic individual-based model for mouse population and analysis of gene drive strategies	Final modeling for locally-fixed alleles manuscript Post-docs now at work in project
3.1.4	Regulatory Engagement	5/1/2017	4/30/2019	5/3/2017	In progress	24%		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	17%		Draft technology scenarios, Workshop report with recommendations, stakeholder map	Interview protocol completed and piloted, stakeholder list for interviews prioritized

Task 3.1.1.1 - Engineer t-Sry mice

- Have derived ES and iPS cells from t-complex mice
- Have received ACURO approval for studies

Task 3.1.1.2 - Generation 1 drive mice

- Zygotic homing unlikely to be viable strategy
- Currently breeding transgenic lines for germline homing experiments

Task 3.1.1.4.1 - Island Selection (included in Jan 2018 update)

Island	Regulatory	*Mus* are non-native	Biosecure	*Mus* are only rodent	Logistics	Amenable to rodenticide treatment	Size and Notes
S.E. Farallon Island	✓	✓	✓	✓	✓	Challenging	39 ha, rodenticide eradication effort stalled
Sand Island, Midway	✓	✓	✓	✓	✓	✓	486ha
Thevenard, Direction Is., W.A.	✓	✓	✓	X	✓	Possible	612 & 24 ha, Forrest's mouse present *(Leggadina forresti)* - not endemic
Boullanger & Whitlock, W.A.	✓	✓	✓	✓	✓	Plans underway for rodenticide treatment	Dibblers and dunnarts present (*Parantechinus apicalis, Sminthopsis boullangerensis)*, 34 ha for Boullanger, 5 ha for Whitlock)
Figure of Eight Is., W.A.	✓	✓	✓	✓	✓	Challenging (Dugites [snake] could ingest mice)	248 ha
Browse Is., W.A.	✓	✓	✓	✓	✓	✓	12 ha (35 acres), need to confirm *Mus* present

Task 3.1.1.4.1 - Island Selection (updated from Jan 2018)

Island	Status and Notes
S.E. Farallon Island	130 mouse samples collected from SE Farallon, 0 from mainland sources thus far
Sand Island, Midway	50 from Midway, ~55 from Oahu sources
Thevenard, Direction Is., W.A.	NCSU IACUC approval of DBCA protocol obtained, but notified by ACURO on 2/6/18 that a separate appendix must be submitted to allow collection by Australian collaborators
Boullanger & Whitlock, W.A.	NCSU IACUC approval of DBCA protocol obtained, but notified by ACURO on 2/6/18 that a separate appendix must be submitted to allow collection by Australian collaborators
Figure of Eight Is., W.A.	NCSU IACUC approval of DBCA protocol obtained, but notified by ACURO on 2/6/18 that a separate appendix must be submitted to allow collection by Australian collaborators
Browse Is., W.A.	NCSU IACUC approval of DBCA protocol obtained, but notified by ACURO on 2/6/18 that a separate appendix must be submitted to allow collection by Australian collaborators
USVI Islands	No updates following hurricanes in September, 2017

Task 3.1.1.4.2 - Locally-Fixed alleles

Provisional Sequencing Plan

- Take 5 samples from each island group and 3-5 samples from each continental group and do whole genome sequencing (WGS; 30X) for high resolution assembly.
- Whole genomes should identify alleles that are fixed 100% on the island and either not present or in only one sample (low frequency) from the continent.
- Follow up with Pool-Seq or targeted re-sequencing (50-100x) for the remaining samples to further characterize the targets identified with WGS.
- Piaggio has been getting estimates for these approaches from partner labs and for-profit labs. Outsourcing appears to be the least expensive have quotes in hand to move forward.
- Post-doc will do library preparation to further reduce costs. Targeted re-sequencing could be accomplished in Piaggio lab.
    - Interviewed postdoc for genomic studies including library preparation and data analysis - experienced candidate with strongly relevant experience

Task 3.1.2 - Systematic and Structured Hazard Analysis

- CSIRO sub-contract with NCSU signed on 16th January 2018
- Genetics team will begin developing systems-based description of Trans-Effector gene drive to provide basis for hazard analysis

Task 3.1.3 - Mathematical modeling

- Final modeling for locally-fixed alleles manuscript
- Initial consideration of modeling of proposed outdoor enclosure (if incorporated in SOW)

Task 3.1.5 - Stakeholder Engagement

- Stakeholder Interview Protocol has been finalized
- List of Stakeholders to be interviewed has been prioritized

Upcoming Tasks

Anticipated work for next reporting period:

- 3.1.1.1: Begin transfecting cell lines with Sry construct
- 3.1.1.2: Breeding transgenic lines to enable germline homing experiment (2-3 months away)
- 3.1.1.3: Optimising PCR screening of off-target sites in ES cells
- 3.1.1.4: Transfer Farallon and Oahu samples, initiate sample preparation, define sequencing strategy
- 3.1.2: Genetics team to develop systems-based description of gene drive as basis for hazard analysis
- 3.1.3: Continued development of individual-based models; Modeling outdoor enclosure if added to SOW
- 3.1.4: No project activities planned for this reporting period
- 3.1.5: Complete stakeholder interviews, Code interviews in Dedoose.com

Restoring Ecosystems and Biodiversity through Development of Safe and Effective Gene Drives: New Tasks in Coming Month

Date: 2/6/2018

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	26%	Engineer t-Sry mice to express Sry under doxycycline control	ACURO approval
3.1.1.2	Generation 1 drive mice	5/1/2017	11/30/18	7/1/2017	In progress	24%	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	20%	Identify population-specific Private Alleles in six mouse island population and adjacent mainland populations	Budgeting and approval process for tissue collections, federal hiring freeze
3.1.2	Systematic and Structured Hazard Analysis	5/1/2017	2/28/19	2/1/2018	2/28/19	1%	Completion of FMEA workshop Completion of GenHAZ workshop Description of Adverse Outcome pathways	Commenced, start delayed by contractual delays.
3.1.3	Mathematical modeling of performance of Genome editors	5/1/2017	2/28/19	6/30/2017	2/28/19	25%	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	24%	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	17%	Draft technology scenarios, Workshop report with recommendations, stakeholder map	N/A

Public Affairs and Public Engagement

- Publications
  - Not directly related to *Safe Genes* project, but special issue of *Journal of Responsible Innovation* on gene drives available online now with numerous performers from our team and others
    - Hayes KR, Hosack GH, Dana GV, Foster S, Ford JH, Thresher R, Ickowicz A, Peel D, Tizard M, De Barro P, Strive T and Dambacher JM (2018). Identifying potential adverse effects associated with the release of gene-drive modified organisms. *Journal of Responsible Innovation*. doi: 10.1080/23299460.2017.1415585
    - see other contributions at **https://tinyurl.com/y8z93cf6 or http://www.tandfonline.com/toc/tjri20/current**
- Meetings
  - Submission of Modeling Gene Drive minisymposium proposal for Society for Math Biol 2018 annual meeting (Sydney, Australia), to include John Marshall (UC Berkeley)
- Items for Public Release
  - None as yet

Compliance

- Animal Use protocols
  - Acuro approval completed for Texas A&M component of project.
  - ACURO protocol for collection of tissue samples by Australian collaborators
    - Notified 2/6/2018 that this would require submission of separate appendix (Godwin advised that should not require veterinary review as existing ACURO protocol already has this, which will hopefully eliminate substantial delay)

Additional Items for Discussion

- FOIA request update: North Carolina and Western Australia requests
- The Convention on Biological Diversity AHTEG has opened an online forum on risk assessment and risk management of gene drives. Forum closes 12th February 2018.
- Stakeholder engagement: Tension between transparency and protection of privacy, with respect to identities of interviewed stakeholders

Detailed spend plan