

Staff Summary for October 13-16, 2026

16. Restricted Species Permit Application (consent)**Today's Item**Information ☐Action ☒

Review and consider a restricted species permit application from Stanford University to possess transgenic tunicates (*Botryllus schlosseri* and *Ciona robusta*), transgenic painted urchins (*Lytechinus pictus*), and transgenic bat stars (*Patiria miniata*) and the Department's recommendation.

Summary of Previous/Future Actions

- Receive notice of application August 12-13, 2026
- **Today, consider action on permit issuance** **October 13-16, 2026**

Background

Pursuant to Section 671.1, subsection (a)(8)(H), when the Department approves a restricted species permit for transgenic aquatic animals, the Commission must review the Department's determination at a regularly scheduled public meeting. The Commission may deny issuance of a permit if it finds the applicant cannot meet regulatory requirements for importation, transportation, possession, and confinement of transgenic aquatic animals.

Consistent with these requirements, at its August 2026 meeting the Commission received the Department's memo and recommendation (Exhibit 1) for a permit amendment request from Stanford University (Exhibit 2) seeking to add additional transgenic aquatic species to its restricted species permit. The proposed additional species include two transgenic tunicates (*Botryllus schlosseri* and *Ciona robusta*), transgenic painted urchins (*Lytechinus pictus*), and transgenic bat stars (*Patiria miniata*). The organisms will be used for biological research at Stanford University's Hopkins Marine Station.

In its transmittal letter, the Department affirms that the containment and security measures outlined in the application meet the requirements specified in Section 671. Staff has reviewed the application and agrees with this assessment.

Significant Public Comments (N/A)**Recommendation**

Commission staff: Allow issuance of the permit by the Department under a motion to adopt the consent calendar.

Department: Allow issuance of a restricted species permit amendment to Stanford University to possess transgenic tunicates (*Botryllus schlosseri* and *Ciona robusta*), transgenic painted urchins (*Lytechinus pictus*), and transgenic bat stars (*Patiria miniata*) for biomedical research.

Exhibits

1. [Department memo, received July 31, 2026](#)
2. [Stanford University application, received July 31, 2026](#)

Staff Summary for October 13-16, 2026

Motion

Moved by _____ and seconded by _____ that the Commission adopts the recommendations for items 16 and 17 on the consent calendar.

MEMORANDUM

Date: July 30, 2026

*Signed original on file;
received July 31, 2026*

To: Melissa Miller-Henson
Executive Director
California Fish and Game Commission

From: Megan Hertel, Director

Subject: Agenda Item for the October Meeting: Approval of Restricted Species Permit Application to Possess Transgenic Species

Stanford University (Stanford) currently has a Restricted Species Permit for 30 species, including seven transgenic aquatic species, and has applied for an amendment to their Restricted Species Permit to possess two species of transgenic tunicates (*Botryllus schlosseri* and *Ciona robusta*), transgenic painted urchins (*Lytechinus pictus*), and transgenic bat stars (*Patiria miniata*). In accordance with California Code of Regulations (CCR), Title 14, Section 671.1(a)(8)(H), all approved applications to possess a transgenic aquatic animal shall be reviewed by the California Fish and Game Commission (Commission) at a regularly scheduled meeting. The Commission may deny the issuance of a permit if it determines that the applicant is unable to meet the regulatory requirements for the importation, transportation, possession, and confinement of transgenic aquatic animals.

The transgenic tunicates, urchins, and bat stars will be used for biological research at Stanford University's Hopkins Marine Station. Stanford has outlined measures in their application materials that fulfill the containment and security requirements specified in CCR, Title 14, Section 671.1(a)(8). California Department of Fish and Wildlife (Department) Marine Region staff have coordinated with the Shellfish Health Lab in reviewing the permit. The Department recommends issuing Stanford a Restricted Species Permit amendment to possess transgenic tunicates, painted urchins, and bat stars.

If you have any questions or need additional information on this matter, please contact Dr. Craig Shuman, Marine Regional Manager at R7RegionalMgr@wildlife.ca.gov.

Attachment: Restricted Species Permit Amendment Application



Melissa Miller-Henson, Executive Director
California Fish and Game Commission
July 30, 2026
Page 2

ec: Chad Dibble, Deputy Director
Wildlife and Fisheries Division

Craig Shuman D.Env., Regional Manager
Marine Region

Kirsten Ramey, Program Manager
Marine Region

Adam Frimodig, Senior Environmental Scientist
Marine Region

Sara Briley, Environmental Scientist
Marine Region

Colleen Burge, PhD, Shellfish Pathologist and Research Scientist
Supervisor
Fisheries Branch



State of California – Department of Fish and Wildlife
2025 RESTRICTED SPECIES PERMIT AMENDMENT REQUEST
DFW 1313b (REV. 01/29/2025) Page 1 of 2

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FEB 11 2026

IMPORTANT! YOU MAY NOT OBTAIN ANIMALS PRIOR TO AMENDMENT APPROVAL

Fees include a nonrefundable three percent (3%) application fee, not to exceed \$7.50 per item. (Section 700.4, Title 14, California Code of Regulations CCR).

FEE: ~~\$78.54~~ (Nonrefundable application fee must accompany this amendment request.)

SEE INSTRUCTIONS ON PAGE 2. TYPE OR PRINT CLEARLY.

FIRST NAME Reese	M.I. S	LAST NAME Zasio	PERMIT NUMBER RD-02352
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BUSINESS NAME Stanford University - Hopkins Marine Station	DAY TELEPHONE 650-725-3882
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LOCATION OF ANIMAL(S) HOUSING: Note: Animals being held at multiple locations require inspection certification by the Department that each of those facilities meet the minimum applicable housing requirements as set forth in subsection 671.1(a)(8)(A-F), Sections 671.3-671.4 and/or 671.7(b), Title 14, of the CCR.

ADDRESS 120 Ocean View Blvd	CITY Pacific Grove	STATE CA	ZIP CODE 93950	COUNTY Monterey
ADDRESS	CITY	STATE	ZIP CODE	COUNTY

List all restricted animals **TO BE ACQUIRED** in the following order: mammals, birds, fish, or reptiles. Group animals by order, family, and species. Use the following letters to denote sex: M=Male, F=Female, and U=Neutered or Unknown. Mark an "X" in the **TO BE ACQUIRED** column for animals to be acquired within the next year. **Unique Identifiers:** Use the following letters to denote unique identifying methods (See Section 671.1(c)(3)(J), Title 14, of the CCR): M=Microchip, T=Tattoo, and A=Alternative Method. Aquaculture and fish permittees: Identify the actual number in the ID number field and identify either W=Weight, V=Volume or C=Count in the method field. Remember to complete the Importation Only Section below for animals being imported into California. For California Residents Only: **All native species obtained from a licensed California Wildlife Rehabilitation Facility require a Native Species Exhibiting Permit.** Contact the License and Revenue Branch at (916) 928-5846 or SPU@wildlife.ca.gov if you need additional information.

COMMON NAME	SCIENTIFIC NAME	ID NUMBER	METHOD	SEX	AGE
StarTunicate	Transgenic Botryllus schlosseri	100	A	E	A
Ciona Robusta	Transgenic Ciona robusta	100	A	E	A
Painted Urchin	Transgenic Lytechinus pictus	500	A	E	A
Bat Star	Transgenic Patiria miniata	500	A	E	A

WILL ANIMALS BE IMPORTED INTO CALIFORNIA? ☒ YES, COMPLETE IMPORTATION SECTION ☐ NO, EXPLAIN:

IMPORT ONLY: COMPLETE THIS SECTION IF YOU ARE IMPORTING ANIMALS INTO CALIFORNIA

LIST SPECIES TO BE IMPORTED Ciona robusta	NUMBER OF ANIMALS 100	ORIGIN (State or Country) Japan
PERSON/BUSINESS SHIPPING ANIMALS Yasunori Sasakura - Ciona Intestinalis Transgenic Line Resources	DAY TELEPHONE 0558-22-6605	
ADDRESS Tennodia 1-1-1	CITY Tsukuba City - Ibaraki Pref.	STATE Japan
		ZIP CODE 305-8577
NAME OF CARRIER FedEx	POINT OF ENTRY INTO CALIFORNIA San Francisco	

I certify under penalty of perjury under the laws and regulations of the State of California that all information on this application is true and correct and I am not violating any city or county laws. I agree to comply with the provisions of Section 671, Title 14, of the CCR. I understand it is unlawful to use or possess a permit which was obtained by fraud or deceit (Fish and Game Code Section 1052b). I understand that in the event that this information is found to be untrue or incorrect, the permit will be considered invalid and must be surrendered where purchased and I will be subject to criminal prosecution. I further understand that failure to comply with the terms and conditions of a permit may result in revocation of current permit and/or denial of future permits. Violation of this section is a misdemeanor, punishable by fine of not more than \$1,000.00, imprisonment in the county jail for not more than six months, or both the fine and the imprisonment. In addition, I may be subject to civil penalties as stated in Fish and Game Code Section 2125.

APPLICANT'S SIGNATURE (Must be in ink) X	DATE February 05, 2026
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FOR DEPARTMENT OF FISH AND WILDLIFE USE ONLY

REVIEWED BY/DATE	TRANSACTION#	ISSUED BY/DATE
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State of California – Department of Fish and Wildlife
2025 RESTRICTED SPECIES PERMIT AMENDMENT REQUEST
DFW 1313b (REV. 01/29/2025) Page 1 of 2

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FEB 11 2023

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ADDRESS	CITY	STATE	ZIP CODE	COUNTY
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APPLICANT'S SIGNATURE (Must be in ink) X	DATE February 05, 2026
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FOR DEPARTMENT OF FISH AND WILDLIFE USE ONLY		
REVIEWED BY/DATE	TRANSACTION#	ISSUED BY/DATE



STANFORD UNIVERSITY SCHOOL OF MEDICINE

DEPARTMENT OF COMPARATIVE MEDICINE • QUAD 7, BUILDING 330, STANFORD, CA 94305-5410
(650) 723-3876 • FAX (650) 725-0940

February 6, 2026

California Department of Fish and Wildlife
License and Revenue Branch
1740 N. Market Blvd.
Sacramento, CA 95834

Attn: License and Revenue Branch

Re: 2025 Restricted Species Permit Amendment Request – Permit Number RD-02352

Please find attached our completed 2025 Restricted Species Permit Amendment Request, Restricted Species Permit No. RD-02352, to include transgenic star tunicates, ciona robustas, painted urchins, and bat stars. Please find included with the amendment request the following documents:

Completed 2025 Restricted Species Permit Amendment Request
Credit Card Authorization Form
List of Species to be Acquired
Statement of Purpose
Resumes of Researcher
Letter of Recommendation
Photos of the enclosures
Hopkins Marge Station Emergency Operations and Disaster Plan
IBC Protocol for Transgenic Echinoderms
USDA Registration Certificate
USDA Email of No Longer Required to Renew Registration
USDA NVAP Approval
Stanford University Assurance Approval D16-00134 (A3213-01)
AAALAC Accreditation

If you have any questions, please feel free to contact me @ 650-725-3882.

Sincerely yours,

A handwritten signature in blue ink, appearing to read "Reese S. Zasio".

Reese S. Zasio
Associate Director, VSC Facilities & Planning
Stanford University
Veterinary Service Center
287 Campus Drive
Stanford, CA 94305-5410

List of Species to be Acquired

Star Tunicate – Transgenic - *Botryllus schlosseri*

Qty 100 – Will be produced in-house at Hopkins Marine Station.

Ciona Robusta – Transgenic

Qty 100 – To be acquired from Ciona Intestinalis Transgenic Line Resources –
University Tsukuba, Tsukuba City – Ibaraki Pref., Japan.

Painted Urchin – Transgenic - *Lytechinus pictus*

Qty 500 – Will be produced in-house at Hopkins Marine Station and/or obtained
from National Sea Urchin Resource Center (NSURC), University of California, San
Diego, La Jolla, CA.

Bat Star – Transgenic - *Patiria miniata*

Qty 500 – Will be produced in-house at Hopkins Marine Station.



Statement of Purpose

Provide a statement of purpose describing in detail the planned use for each animal.

The restricted animals will be used solely for academic research purposes. They are necessary to advance our understanding of embryonic development, larval behavior, metamorphosis, and regeneration in marine invertebrates, which is part of the research mission at the Hopkins Marine Station of Stanford University. Details pertaining individual species are as follows:

Ascidians (*Botryllus schlosseri* and *Ciona robusta*)

The purpose of our research is to identify and characterize tissue-specific adult stem cells in the marine tunicates *Ciona robusta* and *Botryllus schlosseri*. As the closest living invertebrate relatives to vertebrates, these species serve as critical biological models for understanding how stem cells contribute to organ development and regeneration.

Botryllus schlosseri (Colonial Species)

- **Production:** Transgenic *Botryllus schlosseri* animals will be produced in-house. We will perform *in vitro* fertilization and microinjection of zygotes with DNA constructs containing a ubiquitous or tissue-specific promoter (e.g., BHF) driving Green Fluorescent Protein (GFP). The resulting transgenic larvae will be settled onto glass slides and reared to the juvenile/colony stage.
- **Experimental Use:** GFP-labeled candidate stem cells will be isolated and transplanted into non-labeled (wild-type) recipient animals. This allows us to visually track ("lineage trace") the migration, differentiation, and contribution of donor stem cells to the developing organs and vascular systems of the host.
- **Monitoring:** We will monitor these animals through their weekly asexual budding cycles (blastogenesis) to determine how adult stem cells maintain and replace tissues.

Ciona robusta (Solitary Species)

- **Source:** We will use transgenic *Ciona robusta* lines (obtained from the CITRES resource center) that ubiquitously express GFP under promoters such as EF1α.
- **Experimental Use:** These GFP-labeled cells will be isolated and transplanted into non-labeled recipient animals to visually track the migration, differentiation, and contribution of specific stem cells to organ maintenance in a live host.

We will monitor these animals throughout their life history transitions to determine how adult stem cells maintain tissue homeostasis. This study provides a unique comparison between a colonial species (*B. schlosseri*) that undergoes whole-body regeneration every week via asexual reproduction and a solitary species (*C. robusta*) that maintains its body plan as a single, growing organism.

These studies are fundamental for advancing knowledge in regenerative medicine and developmental biology.

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Echinoderms (*Lytechinus pictus* and *Patiria miniata*)

We aim to analyze morphogenesis and cell fate specification during development of echinoderm embryos, specifically sea stars (*Patiria miniata*) and sea urchin (*Lytechinus pictus*). To this end we will generate transgenic animals expressing fluorescent proteins in specific subcellular compartments, e.g. nucleus, under the control of ubiquitous or cell type specific promoters.

Lytechinus pictus

- **Production:** Transgenic *Lytechinus pictus* animals will be produced in-house or obtained from the National Sea Urchin Resource Center (NSURC, University of California, San Diego). For in-house production, we will perform *in vitro* fertilization and microinjection of zygotes with DNA constructs containing a ubiquitous or tissue-specific promoter driving fluorescent proteins (e.g., GFP). The resulting transgenic larvae will be settled onto glass slides and reared to the juvenile/colony stage embryos will be cultured to adulthood in the transgenic facility.
- **Experimental Use:** transgenic embryos, larvae and juveniles will be used for multiple research projects aimed at understanding i) the process of early development, ii) larval behavior and iii) metamorphosis.
- **Monitoring:** detailed protocols for generation and monitoring are attached as a separate document (see ABP protocol).

Patiria miniata

- **Production:** Transgenic *Patiria miniata* animals will be produced in-house. For in-house production, we will perform *in vitro* fertilization and microinjection of zygotes with DNA constructs containing a ubiquitous or tissue-specific promoter driving fluorescent proteins (e.g., GFP). The resulting transgenic larvae will be settled onto glass slides and reared to the juvenile/colony stage embryos will be cultured to adulthood in the transgenic facility.
- **Experimental Use:** transgenic embryos, larvae and juveniles will be used for multiple research projects aimed at understanding i) the process of early development, ii) larval behavior and iii) metamorphosis.
- **Monitoring:** detailed protocols for generation and monitoring are attached as a separate document (see ABP protocol).

Resumes of Relevant Personnel

Provide a resume that provides dates and details documenting you or your full-time employee's qualifying experience caring for restricted animals at a facility engaged in a similar or directly related activity to the permit requested and for the animal(s) to be acquired.

Relevant personnel for ascidian species (*Ciona robusta* and *Botryllus schlosseri*):

Name: Karla Palmeri; Role: Mariculture Specialists; Dates: 1985 - Present.

Name: Kathi Ishizuka; Role: Mariculture Specialists; Dates 1985 - Present.

Both Karla Palmeri and Kathi Ishizuka have maintained the Hopkins Marine Station mariculture facility hosting *Botryllus schlosseri* in continuous operation since 1985. This mariculture facility is globally recognized as the only facility capable of maintaining long-lived *Botryllus* colonies, with some lineages successfully thriving for over 20 years.

Kathi and Karla possess over 35 years of daily, hands-on experience caring for *Botryllus schlosseri*, including the management of seawater circulation and water quality monitoring. They have expert-level experience in *in vitro* fertilization, larval settlement, and colony staging. They developed the specialized feeding and cleaning protocols that allow for 20+ year colony survival. This includes the daily assessment of blastogenetic cycles and the recognition of stress or disease in colonial tunicates. Furthermore, for the past three years, they have successfully maintained *Ciona robusta* within our mariculture system.

Relevant personnel for echinoderm species (*Lytechinus pictus* and *Patiria miniata*)

Name: Vanessa Barone; Role: Assistant Professor; Dates: 2024 - Present.

Vanessa Barone has 15 years of experience working with transgenic aquatic organisms and 7 years of experience working with echinoderms:

- 2010 - 2017: As a graduate student at the Institute of Science and Technology Austria (Klosterneuburg, Austria) Vanessa generated and maintained hundreds of transgenic zebrafish animals (*Danio rerio*).
- 2018 - 2024: as a postdoctoral scholar at Scripps Institution of Oceanography, University of California, San Diego (La Jolla, CA, USA), Vanessa reared embryos of multiple marine invertebrate species and was involved in projects leading to the establishment of laboratory cultures and generation of transgenic *Lytechinus pictus*. She also established the very first laboratory cultures of the sea stars *Patiria miniata*, thereby developing protocols that allow rearing this sea star species to sexual maturity within a laboratory setting.
- 2024 - Present: as an Assistant Professor at the Hopkins Marine Station of Stanford University, Vanessa continue to work with *Lytechinus pictus* and *Patiria miniata*, establishing laboratory cultures of both species within the mariculture facility at Hopkins Marine Station.



Stanford
MEDICINE | Veterinary Service Center

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Date: January 28, 2026

To: State of California – Department of Fish and Wildlife

From: Dr. Stephen A. Felt, DVM, MPH, DACLAM, DACVPM
Professor, Comparative Medicine
Attending Veterinarian

Re: Recommendation for Dr. Vanessa Barone to House Transgenic Echinoderms (*Patiria miniata* and *Lytechinus pictus*)

I am the Attending Veterinarian for the Stanford University Animal Care and Use Program including oversight of the use of animals in research at Stanford University. I recommend that Dr. Vanessa Barone is granted permission to house Transgenic Echinoderms (*Botryllus schlosseri*, *Ciona Robusta*, *Patiria miniata* *Lytechinus pictus*) for use as part of her research here at Stanford University at the Hopkins Marine Station.

Dr. Barone has a Stanford University Administrative Panel on Biosafety (APB) protocol for the use of the transgenic echinoderms. The housing facility is inspected daily by Stanford University Hopkins Marine Station personnel and twice yearly by the Stanford University Institutional Animal Care and Use Committee.

If you have any questions, please feel free to contact me at 650-723-5305 or by email @felt@stanford.edu.





Kenia

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Kenia
Tom

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Karla

Tom

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Karla

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3290	3086
2882	2679
2475	2272
2068	1864
1661	1457
1254	1050

HOPKINS MARINE STATION EMERGENCY OPERATIONS AND DISASTER PLAN

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I. OVERVIEW OF EMERGENCY PLANNING FOR LABORATORY ANIMAL FACILITIES

The purpose of the Department of Comparative Medicine (DCM)/Veterinary Service Center (VSC) Hopkins Marine Center Emergency Operations and Disaster Plan, which operates in conjunction with the Monterey Bay Aquarium which provides all life support (electricity, water, feed), is the following:

- To minimize losses of animals during an emergency
- To always provide appropriate veterinary care/euthanasia
- To allow the facility to sustain or restore normal operations after an emergency
- To enable the facility to recruit outside assistance for animals

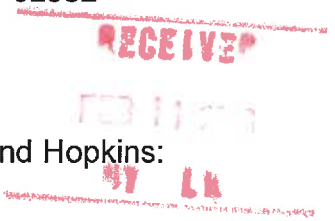
II. DIRECTION AND CONTROL

This plan is designed to protect animals in the event of fire, flood, earthquake, prolonged electrical power outages, or terrorism from animal rights groups. However, it must be stated that the lives and safety of humans within this institution is of top priority. In any emergency, the lives of humans take priority, followed by the lives of research animals.

Alex Norton (agnorton@stanford.edu, (831) 655-6236 (office), 650-(831) 402-5327(cell), Campus Manager, is the Incident Commander and Lucie Hazen (ljhazen@stanford.edu, (831) 655-6239 (cell) serves as backup.

Functions of the Incident Commander:

- Provide information about animals and facilities to Monterey Bay Veterinarian and will also notify the Stanford Attending Veterinarian (or their designee)
- Help set priorities, order evacuation of animals
- Call trained Monterey Bay Aquarium and Campus personnel to assist
- Describe critical resource needs for the animals



III. COMMUNICATIONS

Emergency Notification for emergencies which affect both main campus and Hopkins:

AlertSU and SoM Alert AlertSU

- AlertSU is Stanford University's emergency notification strategy used to communicate time-sensitive information during an emergency event affecting campus. The nature of the incident will determine which of the following methods will be employed to alert the campus community:
- Mass Notification System that sends messages via SMS text message, email, and/or phone to members of the Stanford community
- Outdoor Warning System composed of seven sirens positioned throughout main campus that emit alert tones and verbal instruction intended to reach those who are outdoors

SoM Alert

- Similar to AlertSU, SoM Alert is an emergency notification strategy used to communicate time-sensitive information during an emergency event affecting campus, however, it is targeted to the School of Medicine community.

Stanford Hotline and Emergency Website

- For information during campus-wide emergencies, call the Stanford Emergency Information Hotline (650) 725-5555 or check the Stanford University Emergency Information website (<https://emergency.stanford.edu>).

IV. COMPREHENSIVE RISK ANALYSIS

In the event of a serious disaster (e.g., fire, explosion, massive earthquake), all personnel will proceed to the EAP (Emergency Assembly Point) sign at the parking lot driveway (Attachment 1) and await further instructions.

The Incident Commander (or designee) will ensure that local emergency services (fire department, police, etc.) have been contacted and will contact Stanford University Emergency Response Team (650-725-9999) and the University AV or VOC if animal health or welfare is impacted.

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V. VETERINARY CARE

Once personnel have been approved to enter buildings, every effort will be made to save ALL animals. If resources necessary to continue providing humane care of animals become limited, members of the Hopkins Emergency Response team will prioritize the available resources and preservation of research subjects.

Rank Order of Animal-Related Priorities

Tuna

Other fish spp.

Cephalopods

Other Invertebrates

1. Injured or affected animals will be triaged by trained veterinary staff (Hopkins and/or VSC) if human safety is not compromised. Those animals deemed savable will be treated on site if possible, and/or transported to the Monterey Bay Aquarium Holding Facility (Santa Cruz, 15 miles north of Monterey). Those with life-threatening injuries or conditions not amenable to recovery will be humanely euthanized on site by trained personnel.

2. Humane euthanasia will be performed in accordance with approved APLAC protocols with physical (pithing) and immersion with MS-222 being the two most common methods.



IBC Protocol for Transgenic Echinoderms

Notes

Our experiments involve transposase mediated insertion of exogenous recombinant DNA into the genomes of echinoderms (*L. pictus* and *P. miniata*). No viral DNA, nor a gene drive system is used. Therefore, these experiments fall under section III-D-4-a of the NIH guidelines (April 2024) and require BL-1 containment.

https://osp.od.nih.gov/wp-content/uploads/NIH_Guidelines.pdf

Biosafety levels practice requirements:

<https://ehs.stanford.edu/manual/biosafety-manual/biosafety-level-classification>

Short Summary:

We aim to analyze morphogenesis and cell fate specification during development of echinoderm embryos, specifically sea stars (*Patiria miniata*) and sea urchin (*Lytechinus pictus*). To this end we will generate transgenic animals expressing fluorescent proteins in specific subcellular compartments, e.g. nucleus, under the control of ubiquitous or cell type specific promoters. No viral DNA, nor a gene drive system is used. Therefore, these experiments fall under section III-D-4-a of the NIH guidelines (April 2024) and require BL-1 containment.

Sea star and sea urchin embryos are cultured in filtered seawater. Our laboratory is set up with spaces, equipment and measures to ensure that transgenic echinoderms are not, under any circumstances, released into the wild.

Goals

To visualize morphogenesis and cell differentiation at all stages of embryonic and larval development of selected echinoderm species, i.e. sea stars (*Patiria miniata*) and sea urchin (*Lytechinus pictus*). We will generate stable transgenic lines expressing fluorescent proteins localized to specific subcellular compartments, e.g. nucleus, under the control of ubiquitous or cell-type specific promoters.

Methods

Generation of transgenic echinoderms with transposases

Step 1. Preparation of materials for genome editing

rDNA will be cloned into a plasmid containing Tol2 and/or minos sites using standard cloning techniques (BL-1). Plasmids will be propagated in standard laboratory E.coli strains, e.g. DH1alpha) and isolated via miniprep.

mRNA coding for the relevant transposase (Tol2 or Minos) will be synthesized in vitro from a DNA template (pCS2-Tol2 or pCS2-minos plasmids) using the mMessage capped RNA synthesis kit (Invitrogen).

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Step 2. Delivery of material into early embryos

Oocytes and sperm are obtained by spawning (*L. pictus*) or excising ovaries (*P. miniata*) from WT adults.

A mix of rDNA and transposase mRNA is delivered into oocytes or zygotes via microinjection. This procedure consists of loading small volumes of a mix containing both rDNA and transposase mRNA (2 ul) into a glass needle connected to a precise pressure pump. The needle is then inserted into the oocyte or early embryo and a small volume of mix is injected (in the order of picoliters).

Integration of the rDNA in the genomes of these injected embryos is random and mosaic: not all cells of the animals will carry the transgene. These mosaic individuals are called F0. Once a line has been generated, meaning founders have been identified in which the transgene has integrated in the germline, transgenic embryos will be generated by crossing transgenic adults (F1, F2, etc.). All these transgenic animals are to be handled according to BL-1 standard per NIH guidelines.

Injected embryos are transferred to petri dishes containing seawater and cultured in dedicated incubators for 2-3 days. Subsequently, they are transferred to our larval culture system.

Step 3. Culturing transgenic Larvae

Larvae are free swimming and are cultured inside beakers filled with seawater that is maintained in gentle agitation by a rotating paddle. Larvae are fed daily with a suspension of unicellular algae (*Rhodomonas sp.*). Once a week, the larvae are transferred to a clean beaker with fresh seawater. This larval culturing period lasts 2-6 weeks.

Once larval development is complete, metamorphosis occurs, by which the free swimming larvae transform into sessile juveniles. These juveniles are transferred from the beakers into petri dishes and then into our dedicated juvenile culture system.

Step 4. Culturing transgenic juveniles

Juveniles are cultured in aquaria tanks with continuous flow of sea water: the aquaria are similar to systems used for zebrafish, but are optimized to deal with saltwater.

The juveniles are fed weekly with commercial granular food, e.g. TDO Chroma Boost from Reef Nutrition, and held in their tanks until they are used for experiments, sacrificed or until their death. We expect to keep transgenic animals in their tanks for several years.

Transgenic adults will be used to generate transgenic embryos by spawning (sea urchin) or by dissection of gonads (sea stars).

Precautions

Step 1. Preparation of materials for genome editing

Cloning and isolation of rDNA are performed following BL-1 standards, i.e. we have a dedicated bacterial culture room (Blink 102), all surfaces and tools are disinfected with 70% ethanol after every use and immediately in case of spill, laboratory members use lab coats and gloves as PPEs.

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Synthesis of mRNA coding for transposases (Tol2 or Minos) is performed on a benchtop in the main laboratory space (Blinks 100).

A mix containing both plasmid and transposase mRNA is prepared on a benchtop in the main laboratory space (Blinks 100), following BL-1 standards.

Step 2. Delivery of material into early embryos

Injection of the mix containing plasmid and transposase mRNA into oocytes and early embryos is performed on a dedicated microscope situated in a dedicated space in our lab (part of Blinks 100). The room is not accessible to the public, all equipment, surfaces and tools used are disinfected with 70% ethanol after every use and immediately in case of spills. Microneedles and glass pipettes are collected into biohazard sharp containers.

Integration of the rDNA in the genomes of these injected embryos is random and mosaic: not all cells of the animals will carry the transgene. These mosaic individuals are called F0 and are to be handled according to BL-1 standard per NIH guidelines.

The injected F0 embryos are collected in petri dishes containing seawater, transported into sealed secondary containers and placed into dedicated incubators. 2-3 days after they are transferred to our larval culture system.

Step 3. Culturing transgenic Larvae

The laboratory is equipped with a “larval culture room”, separate from the rest of the laboratory (Blink 103). This room is equipped with a dedicated sink, a custom made shelving unit designed to collect and filter the seawater in which the transgenic larvae are cultured and dedicated biohazard bins (one regular and one for sharps).

Transgenic larvae are cultured in 500 ml glass beakers containing seawater. Each beaker is a self-contained system that can host up to 200 embryos with the same genotype (e.g. F0 injected with the same plasmid). Beakers are placed on a custom made shelving unit: each shelf has a water collection system that guides any spilled seawater into an intermediate tank. Seawater in this tank is then pumped through a filter small enough to catch any embryo or larva (mesh smaller than 100 microns) and the water is then discharged to sanitary. The shelving unit, therefore, acts as secondary containment. When necessary, the filter is exchanged and disposed of in a biohazard bin placed in the larval culture room.

After use, the beakers are washed in DI water and autoclaved. All plasticware and glass pipettes used to transfer transgenic larvae from one beaker to another are disposed of in appropriate biohazard bins (regular for plastic, sharps for glass). All surfaces, tools and equipment, are disinfected with 70% ethanol or with 10% bleach after each use and immediately in case of uncontrolled spills (for instance on the floor).

Step 4. Culturing transgenic juveniles

Hopkins Marine Station is equipped with a dedicated room for transgenic marine animals, (Agassiz 11). To bring our juveniles to that room, we need to cross a courtyard. Therefore, juveniles will be transported while in petri dishes containing sea water and placed into sealed containers.

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The transgenic room has 2 levels of safety:

Level 1: juveniles will be cultured to adulthood in dedicated racks for aquatic animals (Zebtec - Tecniplast), equivalent to those used for zebrafish transgenics. These are closed loop recirculating systems that perform UV sterilization and filtration of outflow water. The outflow seawater is directed to the Level 2 treatment and then back to the ocean.

Level 2: the entire room has a system for collection and treatment of outflow sea water that consists of double (redundant) UV-sterilization and filtration, designed to block any living organisms from being released into the ocean. If both of the redundant systems fail, seawater flow is blocked and excess seawater is diverted to sanitary. This system was designed by a competent contractor (Tenji) in collaboration with the Monterey Bay Aquarium, and has been modelled after the systems used by the Monterey Bay Aquarium to host non-native species.

The level 2 safety system collects water from the Zebtec racks, but also from a floor drain, so that all spills of seawater can be directed through it.

Juveniles and adult transgenic echinoderms will be solely housed in this room, in their dedicated Zebtec racks. Spawning and dissection of gonads will be performed in the transgenic room, to reduce transport of transgenic animals out of the room to a minimum. When needed for examination and experimentation, that might require equipment in other lab spaces, transgenic juveniles and adults will be transported in small tanks with seawater placed into sealed secondary containers.

All surfaces, tools and equipment in the transgenic room will be disinfected with 70% ethanol after every use and immediately in case of spills.

Animal Work

Step 2, 3, and 4 consist of animal work. Procedures and timelines have been summarized in the sections above. Potential for shedding is non-existent, as these transgenics are not generated using viral delivery. The only way in which biological transgenic material can be released in the environment is by accidental spawning of adults, as this results in release of transgenic gametes in the sea water. For this reason, transgenic adults will be housed in a dedicated aquaria system in a dedicated transgenic room, as described in the precaution section of this protocol and reiterated here for your convenience:

The transgenic room has 2 levels of safety:

Level 1: juveniles will be cultured to adulthood in dedicated racks for aquatic animals (Zebtec - Tecniplast), equivalent to those used for zebrafish transgenics. These are closed loop recirculating systems that perform UV sterilization and filtration of outflow water. The outflow seawater is directed to the Level 2 treatment and then back to the ocean.

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Level 2: the entire room has a system for collection and treatment of outflow sea water that consists of double (redundant) UV-sterilization and filtration, designed to block any living organisms from being released into the ocean. If both of the redundant systems fail, seawater flow is blocked and excess seawater is diverted to sanitary. This system was designed by a competent contractor (Tenji) in collaboration with the Monterey Bay Aquarium, and has been modelled after the systems used by the Monterey Bay Aquarium to host non-native species.

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All surfaces, tools and equipment in the transgenic room will be disinfected with 70% ethanol after every use and immediately in case of spills.



United States
Department of
Agriculture

Marketing and
Regulatory
Programs

Animal and
Plant Health
Inspection
Service

Animal Care

EXPIRATION DATE: JUNE 23, 2022

This is to certify that

STANFORD UNIVERSITY

is a registered
under the

CLASS R RESEARCH FACILITY

Animal Welfare Act

(7 U.S.C. 2131 et seq.)

Certificate No. 93-R-0004

Customer No. 1190

Deputy Administrator

RECEIVED

From: [APHIS-AnimalCare](#)
To: [Cheryle E Aird](#)
Subject: RE: [External Email]Query regarding Aphis form 7011 for USDA Registered Facility 93-R-0004
Date: Wednesday, June 1, 2022 1:11:50 PM



Cheryle,

All registered Research facilities are no longer required to renew every three years. However, if there is any contact, site, or other updates that need to be made you should still inform us of those changes so our information matches your institution's most current information.

Best,

USDA Animal Care
2150 Centre Ave. Building B, Mailstop 3W11
Fort Collins, CO 80526-8117



From: Cheryle E Aird <cheryle.aird@stanford.edu>
Sent: Tuesday, May 31, 2022 4:57 PM
To: APHIS-AnimalCare <AnimalCare@usda.gov>
Cc: Cheryle E Aird <cheryle.aird@stanford.edu>
Subject: [External Email]Query regarding Aphis form 7011 for USDA Registered Facility 93-R-0004

[External Email]

If this message comes from an **unexpected sender** or references a **vague/unexpected topic**;
Use caution before clicking links or opening attachments.
Please send any concerns or suspicious messages to: Spam.Abuse@usda.gov

Hello,

As I understand the Animal Welfare Registration (Aphis form 7011) does not need to be renewed every 3 years, would you please let me know if I need to do anything at this time? Our current registration for USDA Registered Facility 93-R-0004 has an expiration date of June 23, 2022. Thank you in advance for any guidance you can offer.

Regards,

Cheryle

Cheryle E Aird, RVT, RLATG, CPIA - Associate Director
Institutional Animal Care & Use Program
Research Compliance Office, Stanford University
1705 El Camino Real

Palo Alto, CA 94306

cheryle.aird@stanford.edu

Phone: 650-736-7065 C: 510-688-1144 F: 650-736-7077

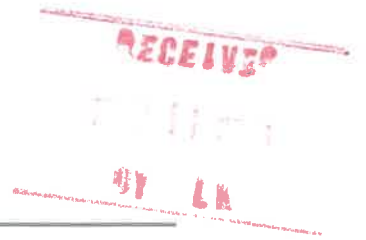
<http://labanimals.stanford.edu>



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From: [Dr. Stephen A. Felt DVM, MPH, DACLAM, DACVPM](#)
To: [Reese S Zasig](#)
Subject: Fwd: USDA NVAP Application Processed
Date: Monday, June 3, 2024 2:51:21 PM



FYI

Sent from my iPhone

Begin forwarded message:

From: vspsAdmin@aphis.usda.gov
Date: June 3, 2024 at 1:30:23 PM PDT
Subject: USDA NVAP Application Processed

National Accreditation Number: 044323
Accreditation Renewal Date: 07/31/2027
Accreditation Category: II

Dear Dr. Felt:

This email is to inform you that the application you recently submitted has been approved. Please note your renewal date above. No further action is required by you at this time. This is the only notification you will receive for the successful processing of this application.

Please remember to continue to keep your contact information up to date. For directions on how to update your contact information in the future, please visit the NVAP website at: www.aphis.usda.gov/nvap/ or contact your VS NVAP Coordinator.

To locate your VS NVAP Coordinator, go to:
<https://www.aphis.usda.gov/aphis/ourfocus/animalhealth/nvap/nvap-coordinators>

National Veterinary Accreditation Program



DEPARTMENT OF HEALTH & HUMAN SERVICES

PUBLIC HEALTH SERVICE
NATIONAL INSTITUTES OF HEALTH

FOR US POSTAL SERVICE DELIVERY:

Office of Laboratory Animal Welfare
Division of Assurances
6700B Rockledge Drive
Suite 2500, MSC 6910
Bethesda, Maryland 20892-6910
Home Page: <https://olaw.nih.gov>

FOR EXPRESS MAIL:

Office of Laboratory Animal Welfare
Division of Assurances
6700B Rockledge Drive, Suite 2500
Bethesda, Maryland 20817
Telephone: (301) 496-7163

07/22/2025

Re: Approval for D16-00134 (A3213-01)

David Studdert, LLB, Sc.D.
Vice Provost and Dean of Research
Stanford University (Leland Stanford Junior University)
1705 El Camino Real
Palo Alto, CA 94306

Dear Dr. Studdert:

I am pleased to inform you that the Office of Laboratory Animal Welfare (OLAW) reviewed and approved your institution's Animal Welfare Assurance (Assurance) that was submitted in accordance with the Public Health Service (PHS) Policy on Humane Care and Use of Laboratory Animals (Policy), revised 2015. Your Assurance, identification number D16-00134 (A3213-01), became effective on 07/22/2025 and will expire on 05/31/2029. Please include the Assurance number on all correspondence to OLAW. A copy of the signed Assurance document will be sent in a separate email. The signature page provides verification of approval by OLAW and includes the period during which your institution's Assurance is effective. The Assurance is a key document that sets forth the responsibilities and procedures of your Institution regarding the care and use of laboratory animals according to the PHS Policy. The practices described in the Assurance document must be followed by all individuals in the animal care and use program.

Please note that the OLAW Annual Reporting period is the federal fiscal year, October 1 through September 30. The Annual Report to OLAW is due by December 1 (but no earlier than September 30). If I may be of any further assistance, please do not hesitate to contact me.

Sincerely,

Animal Welfare Program Analyst, OLAW
National Institutes of Health

cc: IACUC Contact
POC



June 16, 2025

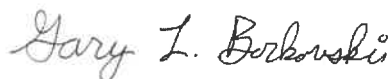
Cheryle E. Aird, RVT, CPIA, RLATG
Associate Director, Animal Care and Use Program
Research Compliance Office
Stanford University
1705 El Camino Real
Palo Alto, CA 94306

Dear Ms. Aird:

AAALAC International is pleased to convey that the Leland Stanford Junior University, Stanford, California is accredited. Our records show that Leland Stanford Junior University initially achieved AAALAC International accreditation on June 27, 1988.

This interest, contribution and participation in the AAALAC International accreditation program is valued and appreciated. As you are aware, AAALAC International conducts site visits to institutions and requires being apprised of significant programmatic changes during the interim between these site visits. Should you desire additional information or wish to comment on any aspect of the accreditation process, please contact our office.

Sincerely,

A handwritten signature in dark ink that reads "Gary L. Borkowski". The signature is written in a cursive, flowing style.

Gary L. Borkowski, D.V.M., M.S.
Chief Executive Officer

GLB:mem
000679