

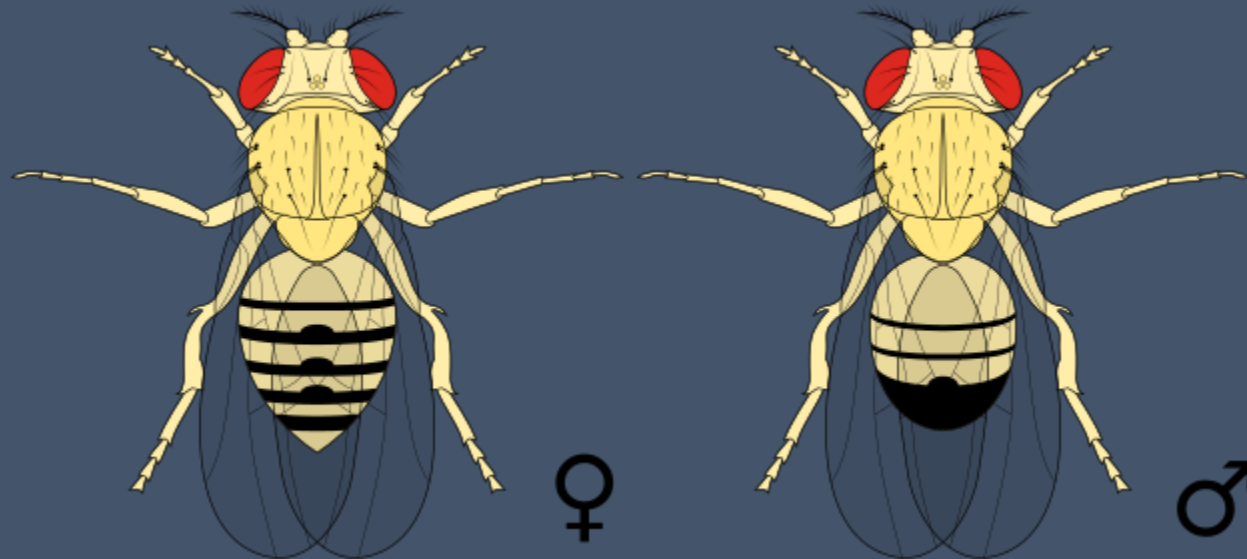
**RISK ASSESSMENT  
AND FACILITY CONTAINMENT FOR RESEARCH  
WITH GENE DRIVE ARTHROPODS**

Sheryl Major  
Biosafety Officer

UC San Diego

# Proposed Research Project:

Researcher proposes to use gene drive tool in a live arthropod model



Ask yourself, is your arthropod...



Indigenous



Pest



Vector

# What infrastructure do you need in your lab?

## Life Support

- Temperature, Humidity, Life Cycles, Light Cycles

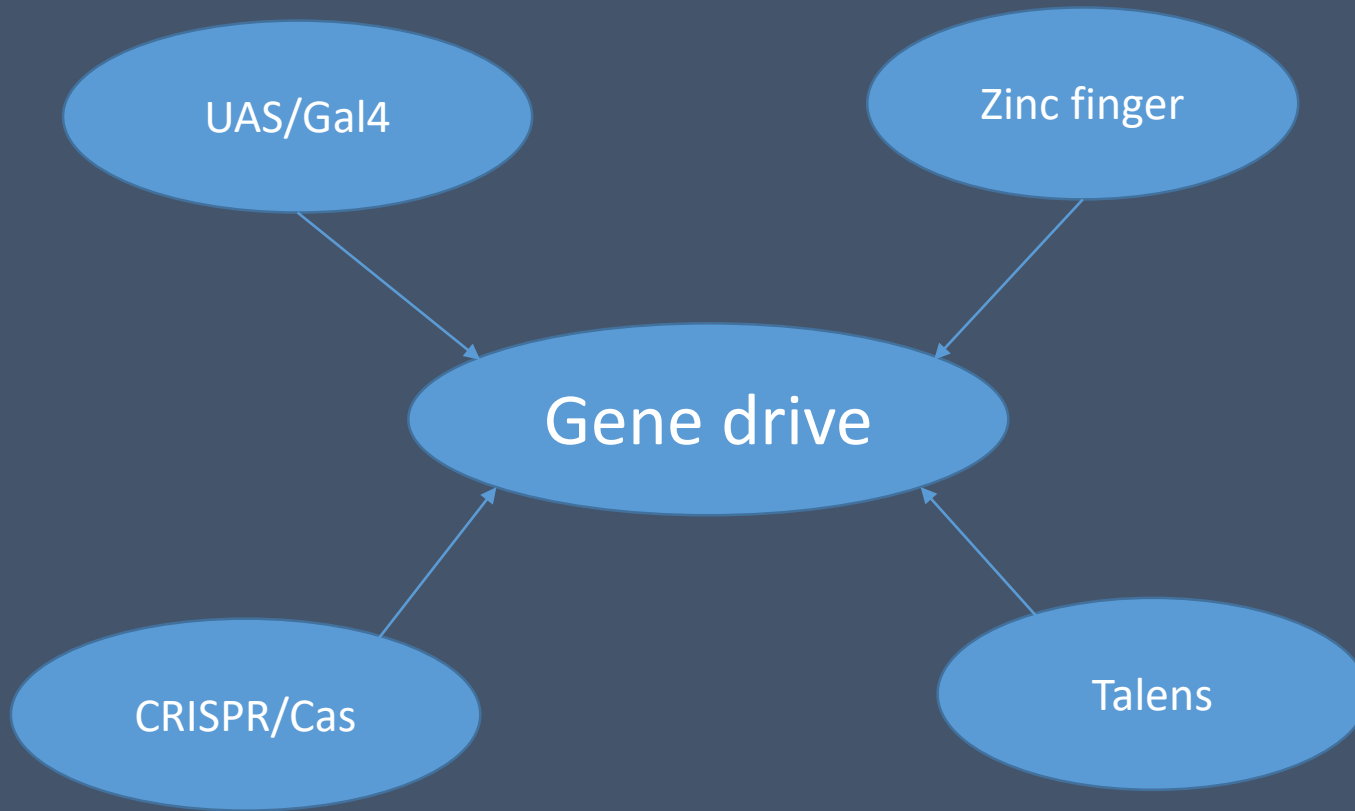
## Handling

- Microinjections, Microscopy, Feeding, Euthanasia

## Containment Needs

- Mobility, Survivability, Waste Disposal, Trapping

# Gene Drive



Gene drives are systems that ensure biased inheritance by enhancing the likelihood a sequence of DNA passes between generations through sexual reproduction and potentially throughout an entire population.

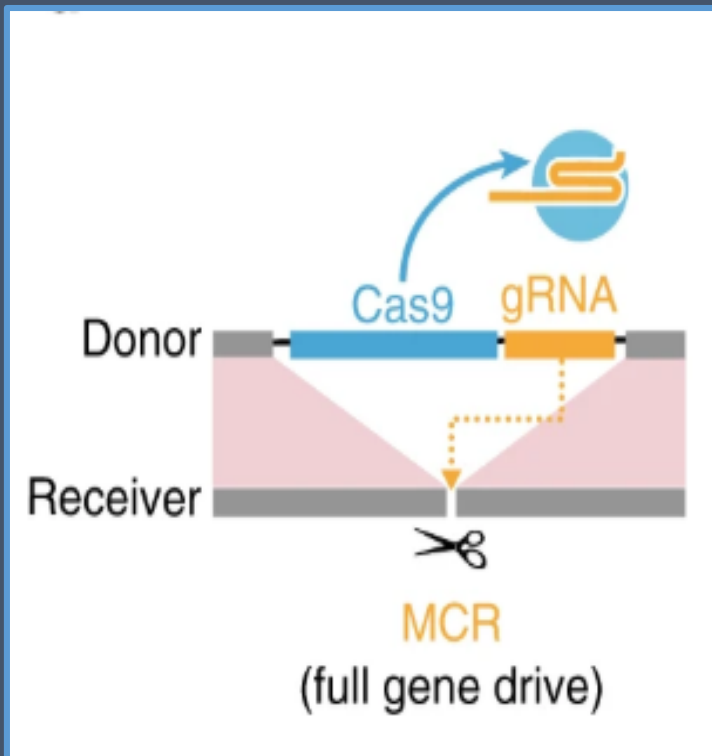
**ZFN, TALEN, and CRISPR/Cas-based methods for genome engineering.**

[Gaj T Gersbach CA Barbas CF 3rd https://www.ncbi.nlm.nih.gov/pubmed/23664777](https://www.ncbi.nlm.nih.gov/pubmed/23664777)

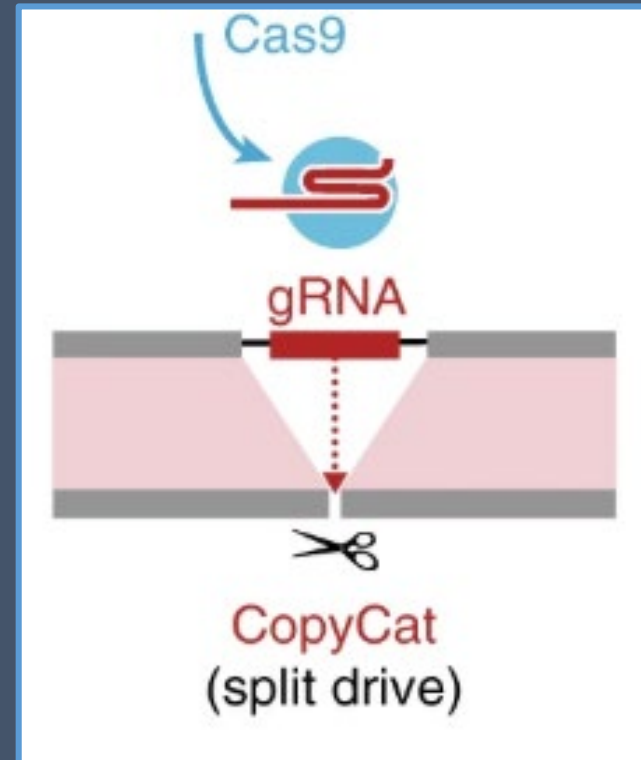
# CRISPR/Cas9 as a Gene Drive

## Molecular Containment

### Mutagenic Chain Rxn



### Split Drive



Efficient allelic-drive in *Drosophila* [<https://www.nature.com/articles/s41467-019-09694-w> <http://creativecommons.org/licenses/by/4.0/>]

# What tools can I use for my risk assessment?



## Containment Facility Inspections

Last Modified: Jun 19, 2019



APHIS has developed guidelines for the containment of organisms. These guidelines vary depending upon the type of organism to be contained and the risk posed by those organisms. **Not all elements in the following guidelines apply to all organisms to be contained and the guidelines are guidelines not regulations.**

- [Containment Guidelines for Educational Displays of Adult, Butterflies and Moths \(Lepidoptera\)](#)
- [Containment Facility Guidelines for Noxious Weeds and Parasitic Plants](#)
- [Containment Guidelines for Non-Indigenous, Phytophagous Arthropods and Their Parasitoids and Predators](#)
- [Containment Guidelines for the Receipt, Rearing and Display of Non-Indigenous Arthropods in Zoos, Museums, and Other Public Displays](#)
- [Containment Guidelines for Plant Pathogenic Nematodes](#)
- [Containment Guidelines for Non-Indigenous Snails](#)
- [Containment Guidelines for Plant Pathogenic Bacteria](#)
- [Containment Facility Guidelines for Viral Plant Pathogens and Their Vectors](#)
- [Containment Facility Guidelines for Fungal Plant Pathogens](#)

## CDC Import Permit Inspection Checklist for Arthropod Containment Level 2 (ACL-2)

Entity Name: \_\_\_\_\_ Inspection Date: \_\_\_\_\_  
 Street Address: \_\_\_\_\_  
 City, State, Zip: \_\_\_\_\_  
 Lead Inspector: \_\_\_\_\_  
 Other Inspectors: \_\_\_\_\_  
 Building/Room(s): \_\_\_\_\_  
 PI(s): \_\_\_\_\_

Entity Name:		Inspection Date:			
Reference	Statement	Yes	No	N/A	Comments
CFR: 71.54 (b)	<b>Unless excluded pursuant to paragraph (f) of this section, a person may not import into the United States any infectious biological agent, infectious substance or vector unless:</b>				
CFR: 71.54 (b)(1)	It is accompanied by a permit issued by CDC. The possession of a permit issued by CDC does not satisfy permitting requirements placed on materials by the U.S. Department of Agriculture that may pose hazards to agriculture or agricultural production in addition to hazards to human health.	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
CFR: 71.54 (b)(2)	The importer takes measures to help ensure the shipper complies with all permit requirements and conditions.	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
CFR: 71.54 (b)(3)	The importer has implemented biosafety measures commensurate with the hazard posed by the infectious biological agent, infectious substance, and/or vector to be imported, and the level of risk given its intended use.	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	

USDA Containment Facility Inspections [<https://www.aphis.usda.gov/aphis/ourfocus/planthealth/import-information/permits/plant-pests/containment>]

CDC ACL-2 Inspection Checklist [<https://www.cdc.gov/cpr/ipp/inspection/index.htm>]

# What tools can I use for my risk assessment?

## Arthropod Containment Guidelines

The screenshot shows the title page of the 'Arthropod Containment Guidelines, Version 3.2'. The title is prominently displayed at the top. Below the title, the authors are listed as the 'American Committee of Medical Entomology; American Society of Tropical Medicine and Hygiene'. There are links for 'Author information', 'Copyright and License information', and 'Disclaimer'. A yellow highlighted box contains the text: 'This article has been cited by other articles in PMC.' Below this is the 'Abstract' section, which begins with: 'The Arthropod Containment Guidelines are a product of the work of the American Committee of Entomology, a subcommittee of the American Society of Tropical Medicine and Hygiene. The guidelines provide a reference for research laboratories to assess risk and establish protocols for the safe handling of BSL-2 agents associated with animal and/or human disease, or that are reasonably suspected of being infected with such agents (diagnostic samples). The guidelines provide a reference for research laboratories to assess risk and establish protocols for the safe handling of BSL-2 agents associated with animal and/or human disease, or that are reasonably suspected of being infected with such agents (diagnostic samples). The guidelines provide a reference for research laboratories to assess risk and establish protocols for the safe handling of BSL-2 agents associated with animal and/or human disease, or that are reasonably suspected of being infected with such agents (diagnostic samples).'

## Gene Drive Containment Plan

The screenshot shows a web form titled 'Containment Plan for Research with Gene Drives'. The form is partially filled out. It includes sections for '1. What is the function of the gene drive?', '2. What is the function of the gene drive?', '3. What are the containment strategies...', and '4. What are the containment strategies...'. There are several checkboxes and input fields. The form also includes a 'PI Name' field with sub-fields for 'First', 'Middle', and 'Last', and a 'BUA Number' field. The form is dated 'Last Updated: December 19, 2018 8:24:30 AM PST'.

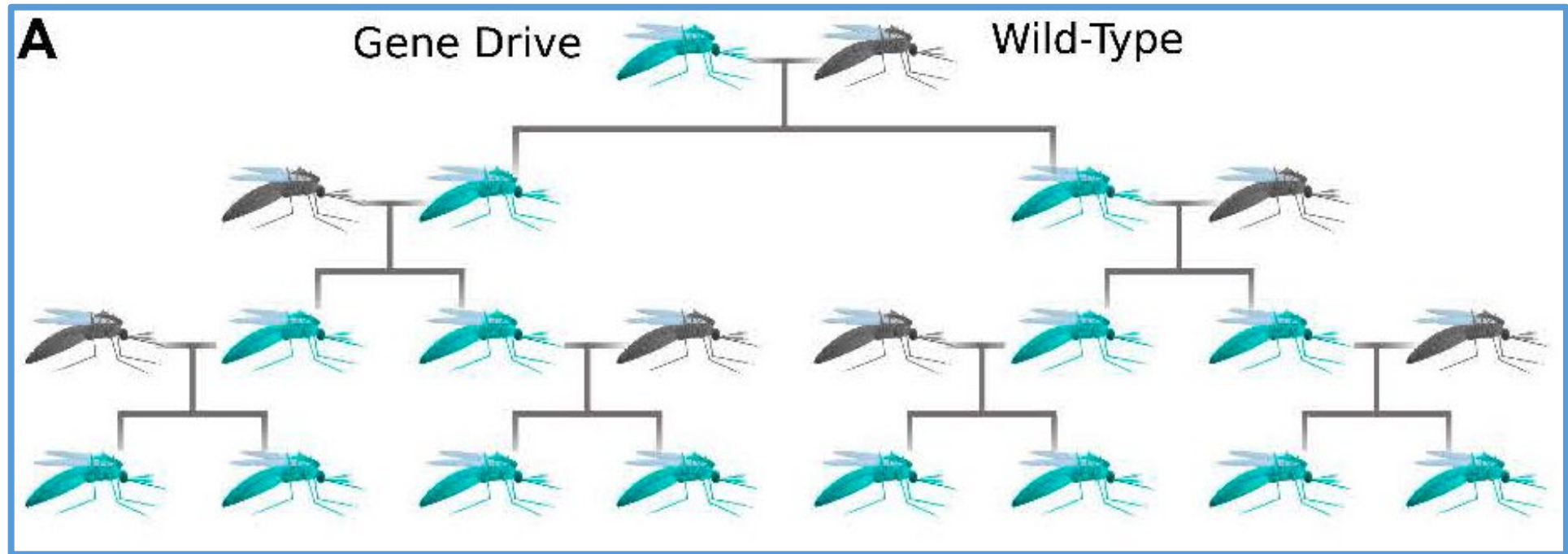
UC San Diego Gen Drive Containment Plan [<https://blink.ucsd.edu/sponsor/EHS/forms-ehs/containment-plan-gene-drives.html>]

Arthropod Containment Guidelines [<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6396570/>]

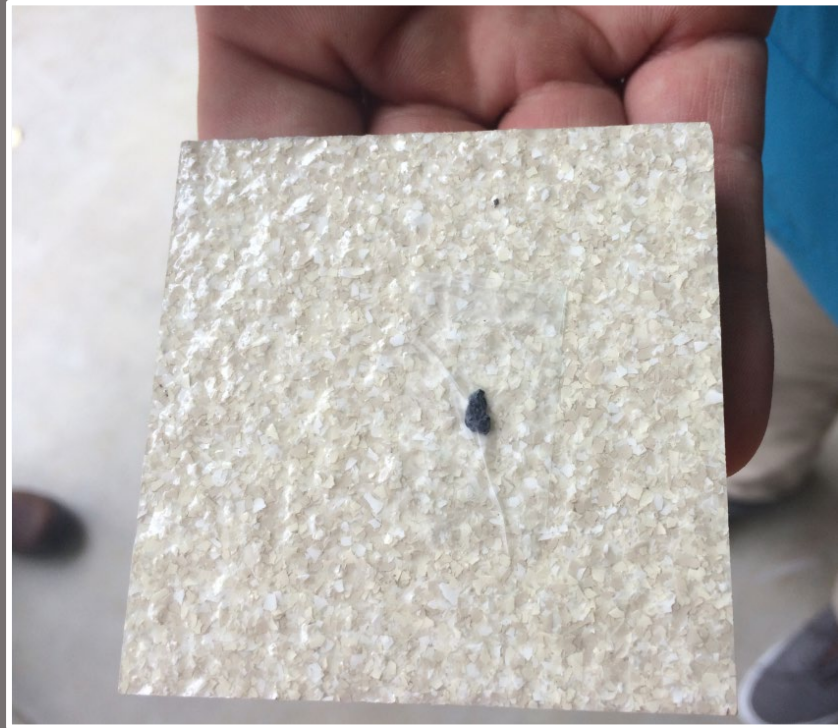
EU Horizon 2020 Guidelines for CL2/3 [<https://infravec2.eu/wp-content/uploads/2018/12/Insectary-Design-Infravec-2-Final-Version-1.1-2.pdf>]



Researcher proposes to use CRISPR/Cas9 MCR gene drive tool in germline cells of a live *Aedes aegyptii* at Arthropod Containment Level 2 (ACL-2)



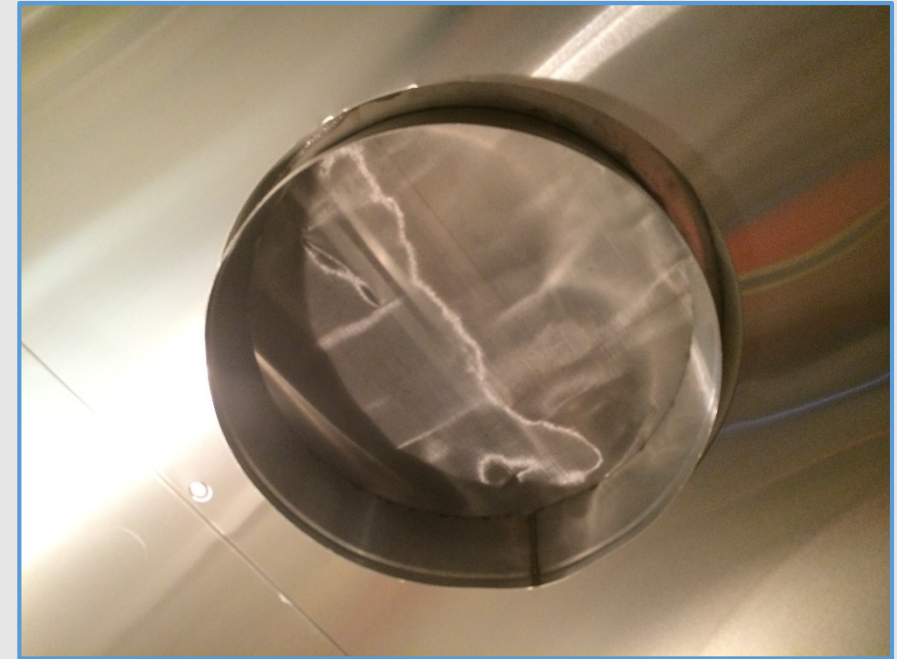
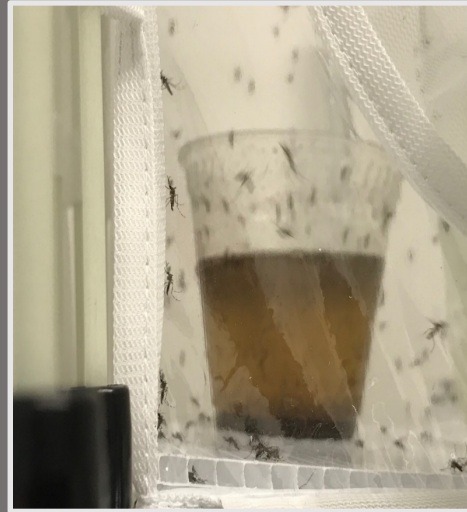
Is that dirt or a  
Mosquito?



How do you capture all  
these swimming pupae if  
the container spills?

UC San Diego

What mesh size contains the flying stage?



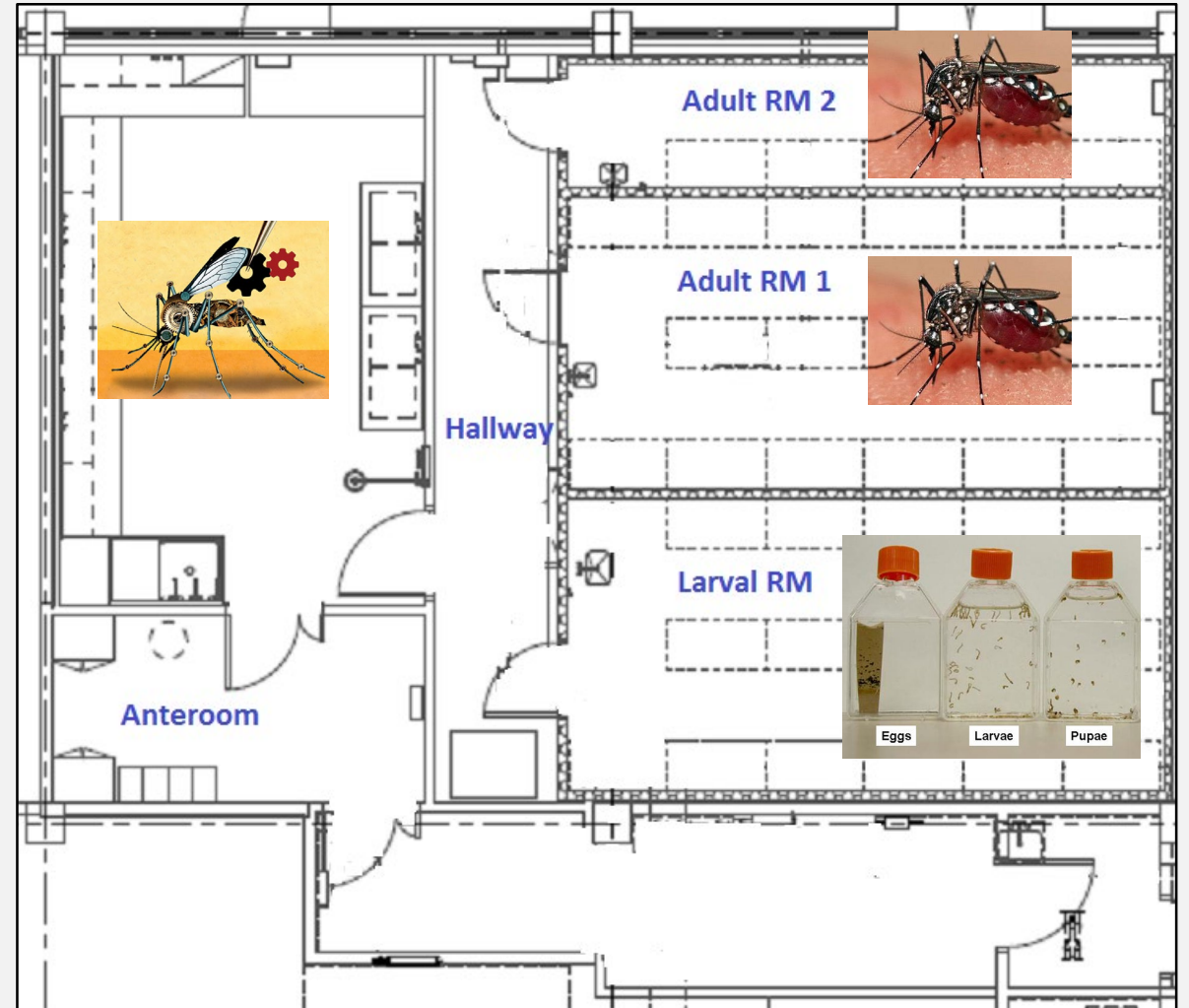
Will the mesh cover on the Exhaust register clog with dirt?

# Do you need an Effluent Decontamination System (EDS)?

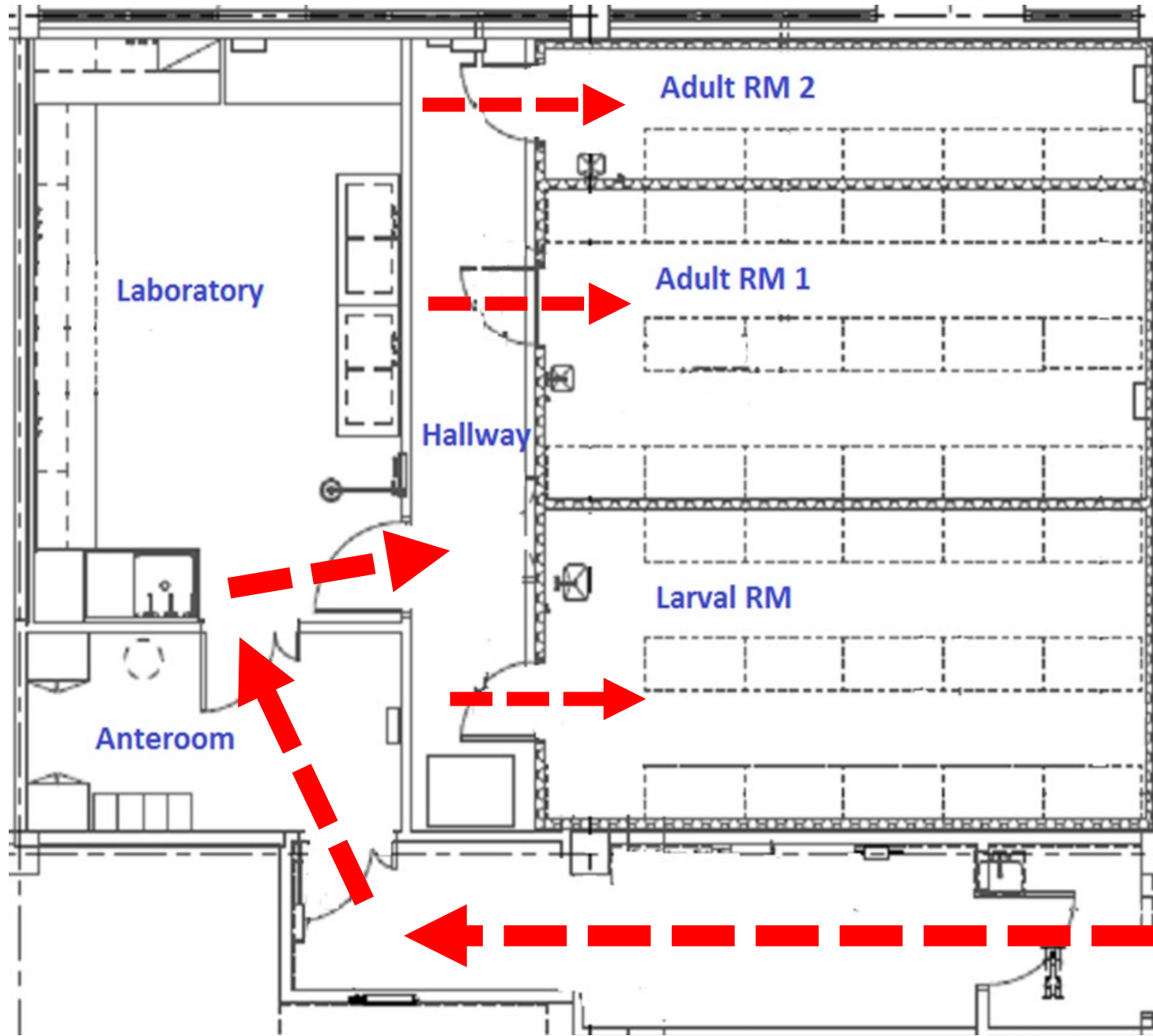




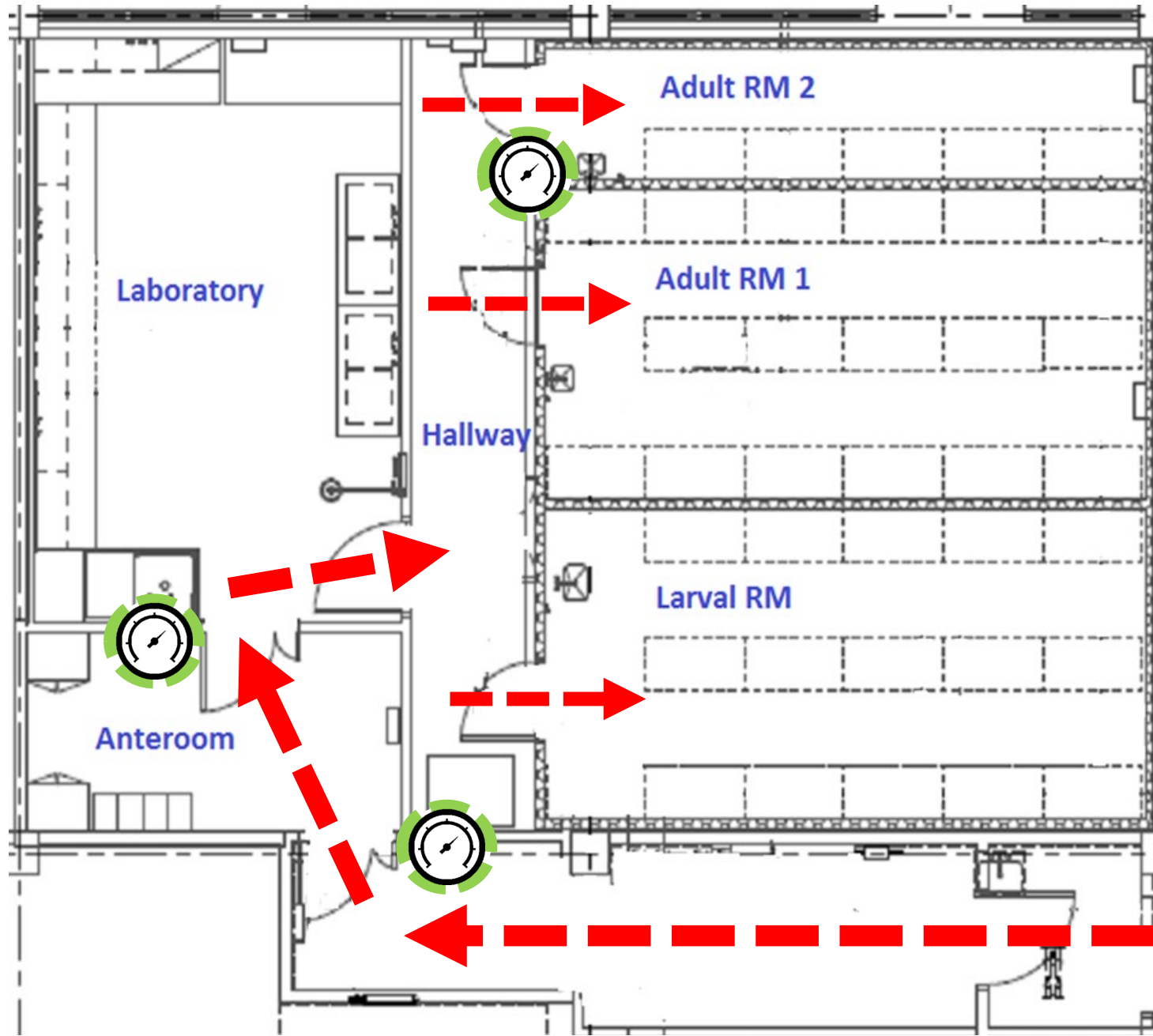
# MOSQUITOES ACL-2



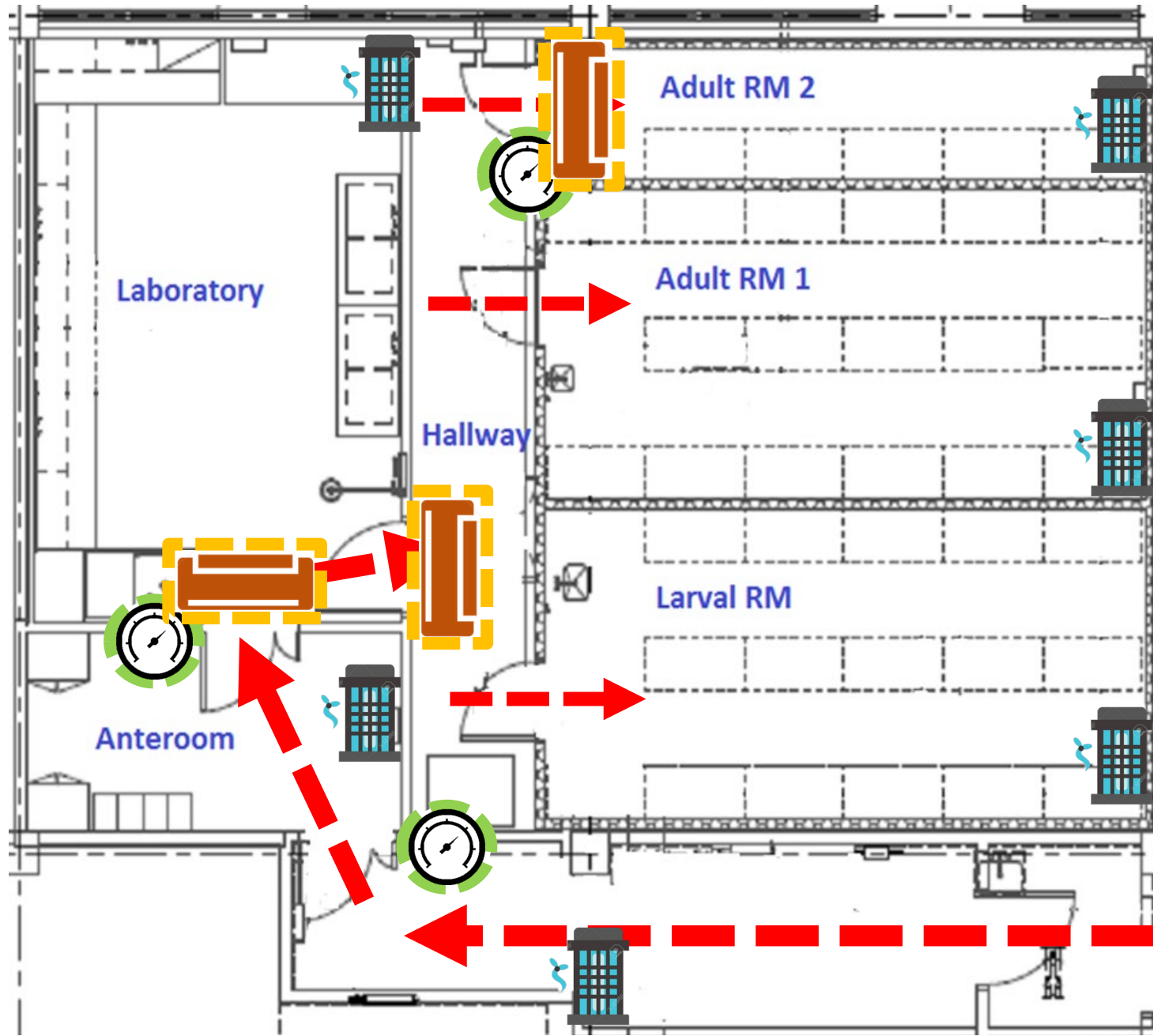
Air Flow



# Diff. Pressure Monitors

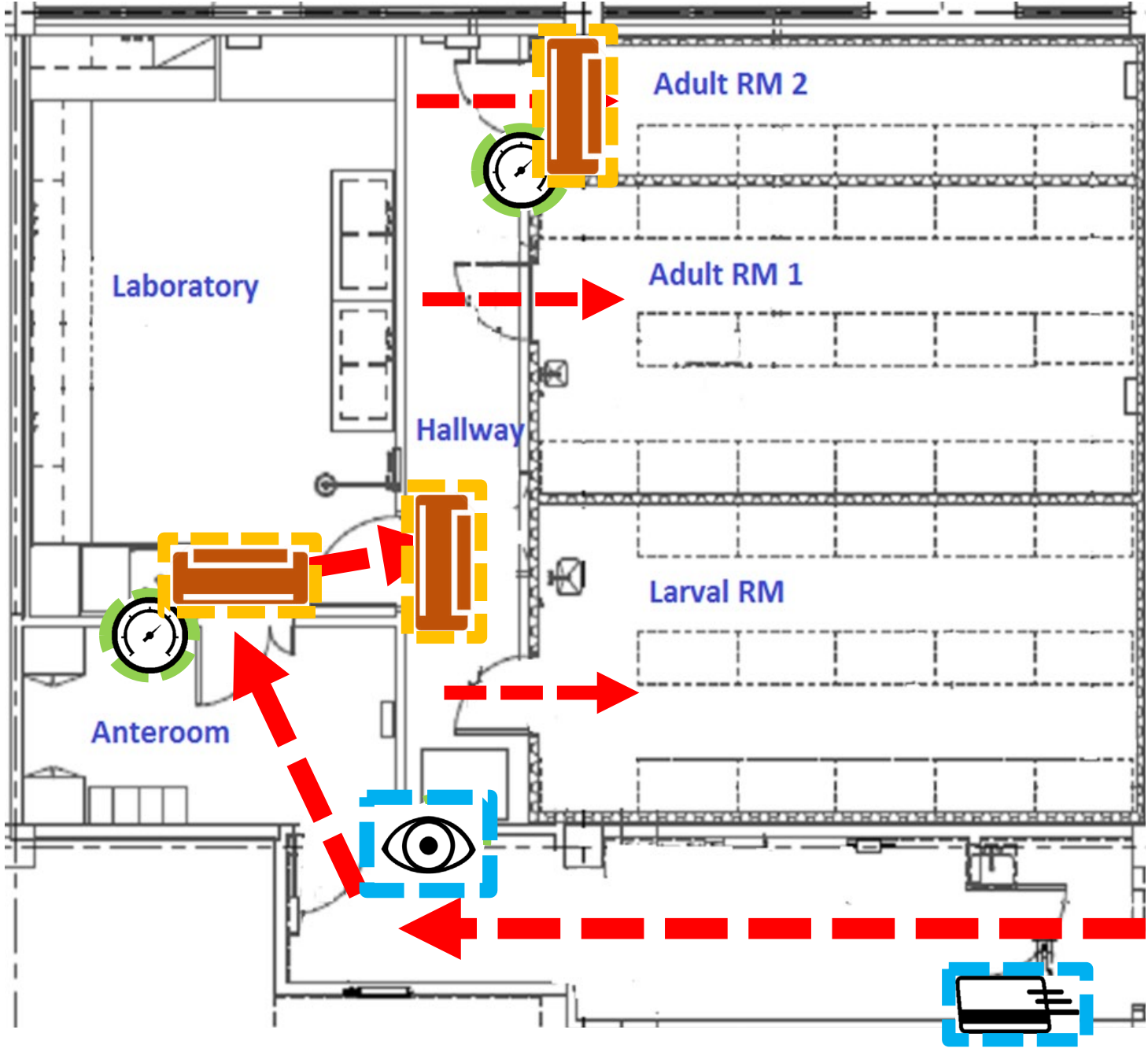


# Air Curtains

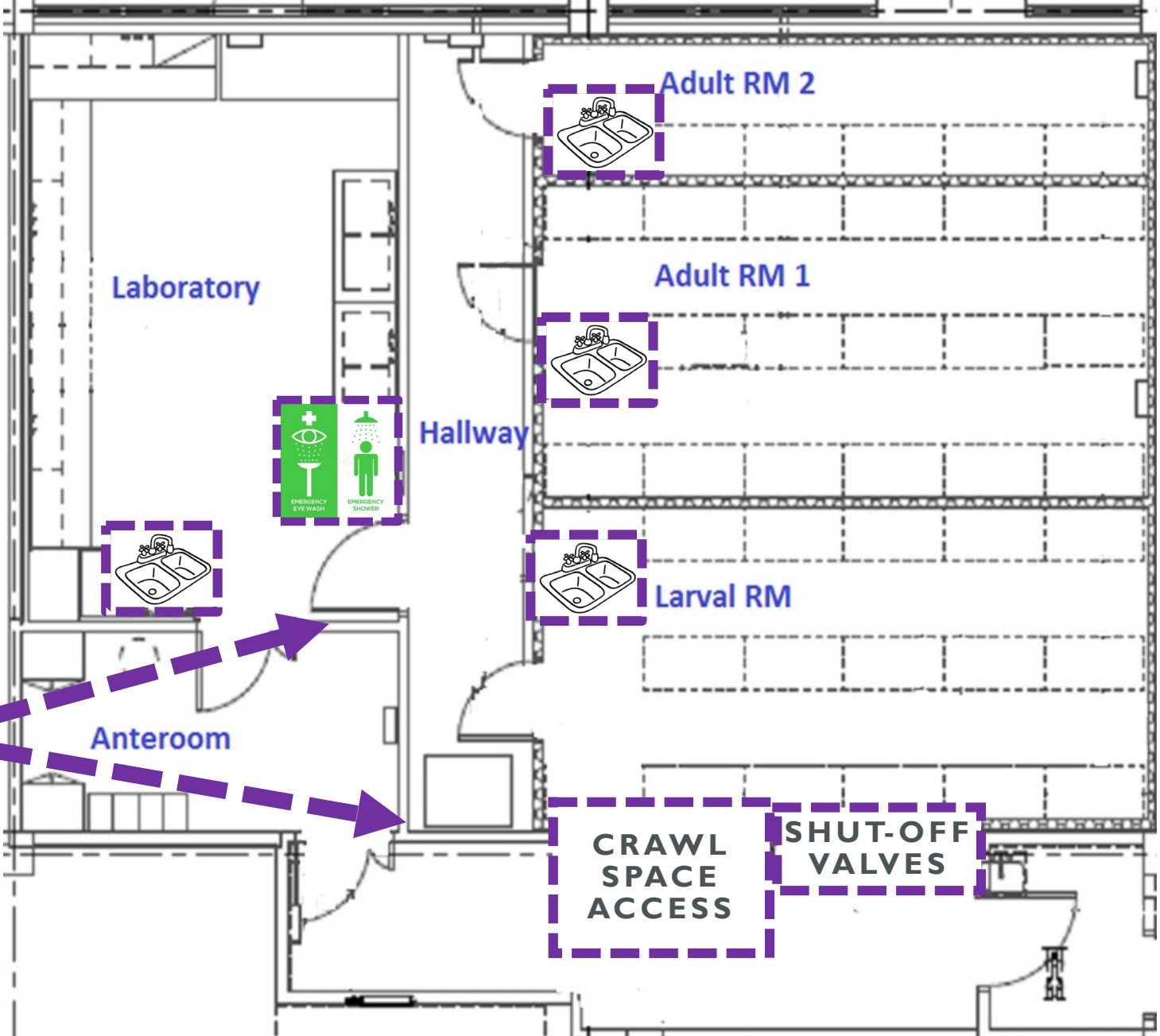




# Security

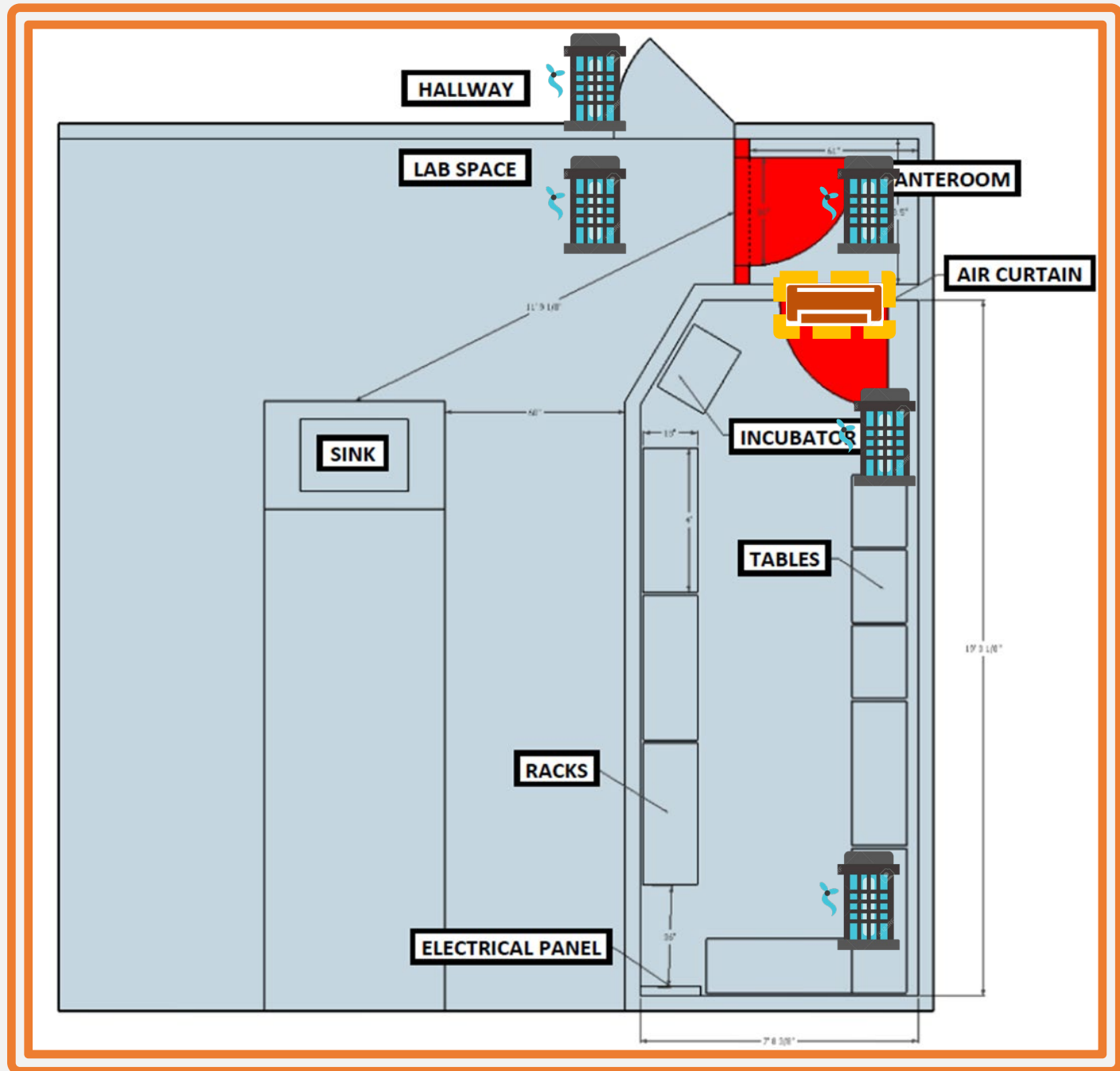


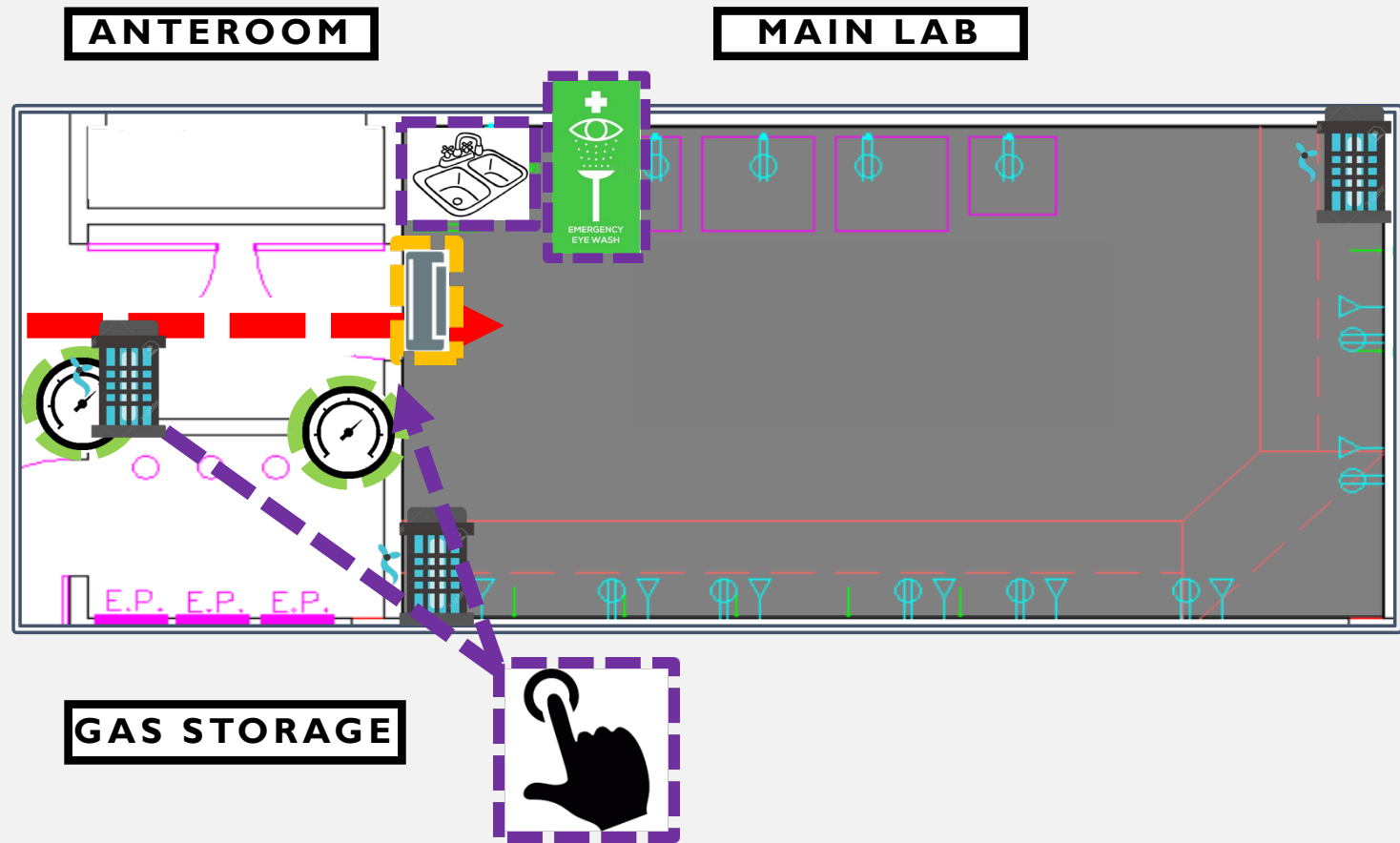
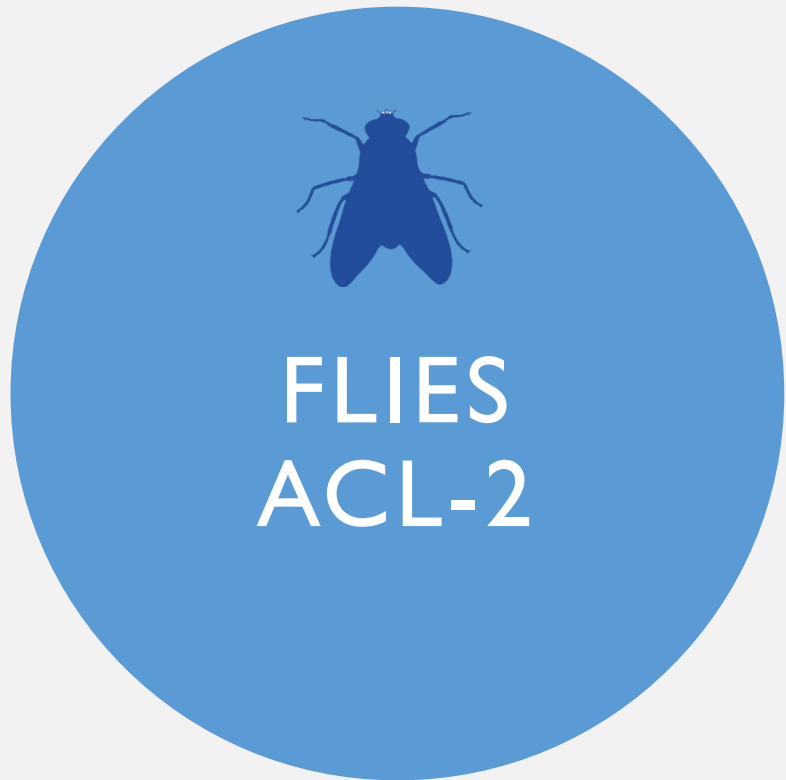
# Emergency Response





# MORE MOSQUITOES ACL-2





# What will work for your Institution?

## References:

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American Committee Of Medical Entomology American Society Of Tropical Medicine And Hygiene. Arthropod Containment Guidelines, Version 3.2. *Vector Borne Zoonotic Dis.* 2019;19(3):152–173. doi:10.1089/vbz.2018.2431

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UC San Diego