

Insect Genetic Technologies

Why, How

Biosafety Challenges

David O'Brochta, Ph.D.

Institute for Bioscience and Biotechnology Research

Department of Entomology

University of Maryland, College Park

dobrocht@umd.edu



Insect Genetic Technologies:

Genetic Ablation Analysis	
Homologous Recombination-based Transgene Integration - ZFN, TALEN	
Mis-expression Analysis	
Site-specific Recombination-based Deletions and Inversions, e.g. Flp/FRT	
Editing & Knockouts - ZFNs, TALENs, CRISPR/Cas	
Transposon-based Sensor Systems, e.g. enhancer-trap	
Modular Gene Expression Systems, e.g. Gal4	
Transgenic RNAi Gene-Silencing	
Site-specific Recombination-based Transgene Integration - ΦC31	
Transposon-based Transgene Integration	
dsRNA Gene Silencing	
<i>Drosophila melanogaster</i>	
<i>Bombyx mori</i>	
<i>Aedes aegypti</i>	
<i>Anopheles stephensi</i>	
<i>Anopheles gambiae</i>	
<i>Anastrepha suspensa</i>	
<i>Ceratitis capitata</i>	
<i>Tribolium castaneum</i>	
<i>Gryllus bimaculatus</i>	
<i>Lucilia cuprina</i>	
<i>Nasonia vitripennis</i>	
<i>Drosophila sp.</i>	
<i>Apis mellifera</i>	
<i>Harpegnathos saltator</i>	
<i>Bicyclus anynana</i>	
<i>Chironomus riparius</i>	
<i>Cochliomyia hominivorax</i>	
<i>Cochliomyia macellaria</i>	
<i>Culex quinquefasciatus</i>	
<i>Danaus plexippus</i>	
<i>Glossina morsitans</i>	
<i>Lucilia serricata</i>	
<i>Manduca sexta</i>	
<i>Mayetiola destructor</i>	
<i>Megaselia abdita</i>	
<i>Musca domestica</i>	
<i>Oncopeltus fasciatus</i>	
<i>Acyrtosiphon pisum</i>	
<i>Heliconius sp.</i>	
<i>Diaphorina citri</i>	
<i>Bactericera cockerelli</i>	
<i>Clogmia albipunctata</i>	
<i>Coboldia fuscipes</i>	
<i>Culex quadrimaculatus</i>	
<i>Culex tarsalis</i>	
<i>Culicoides sonorensis</i>	
<i>Polistes dominulus</i>	
<i>Polistes fuscatus</i>	
<i>Polistes metricus</i>	

Why?

Research

Functional Genomics

Applications

Physically Contained

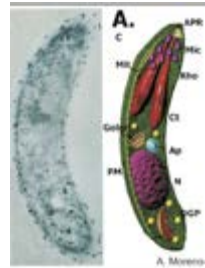
Genetically Contained

Uncontained

Research Functional Genomics



Salivary Gland

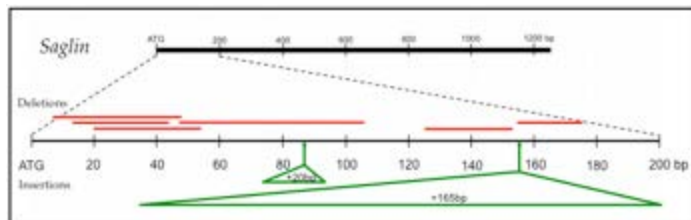
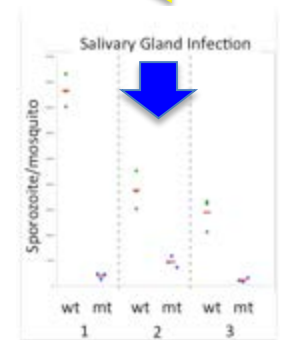
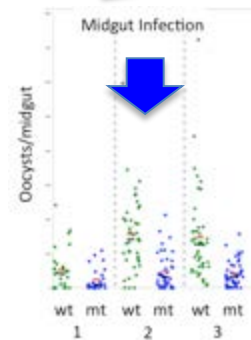
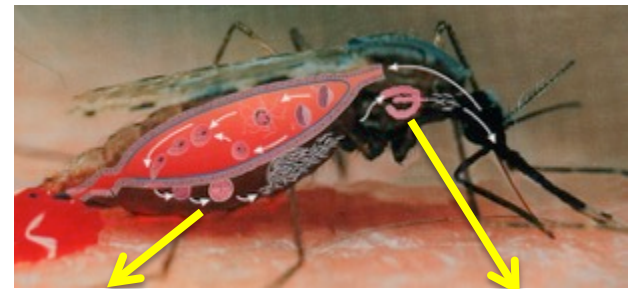
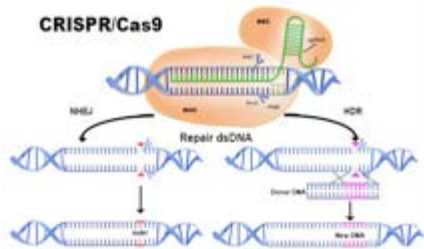


Sporozoite

Saglin

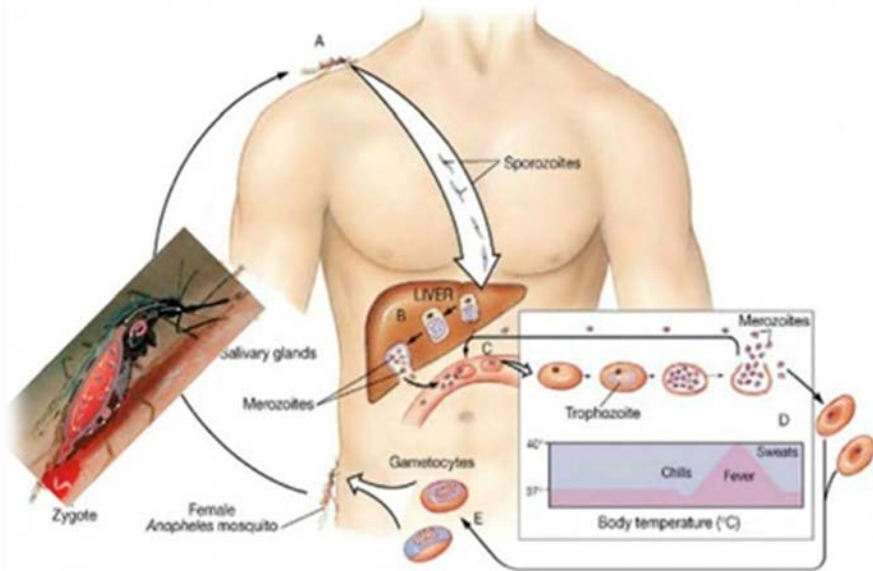


CRISPR/Cas9 Mutants



Physically Contained Applications

Genetically Modified Mosquitoes and a Malaria Vaccine

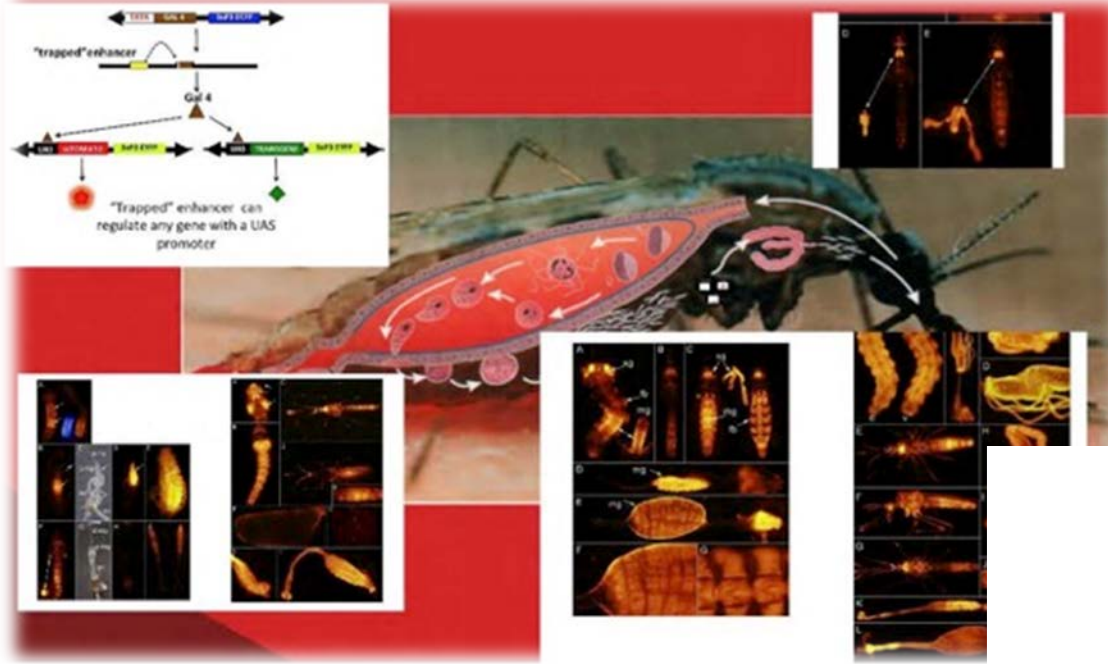


Sanaria, Inc.

The composite image features a mosquito with a green box highlighting its head, a yellow radiation warning symbol with the word "RADIATION" below it, a test tube containing a green liquid, and a vaccine vial. A green arrow points from the mosquito to the test tube, and another green arrow points from the test tube to the vaccine vial. A red prohibition sign is overlaid on the human host diagram from the previous image.

New malaria vaccine shows prom
Barbara Mantel, NBC News
Aug. 8, 2013 at 2:04 PM ET
After decades of frustrating research to find a highly

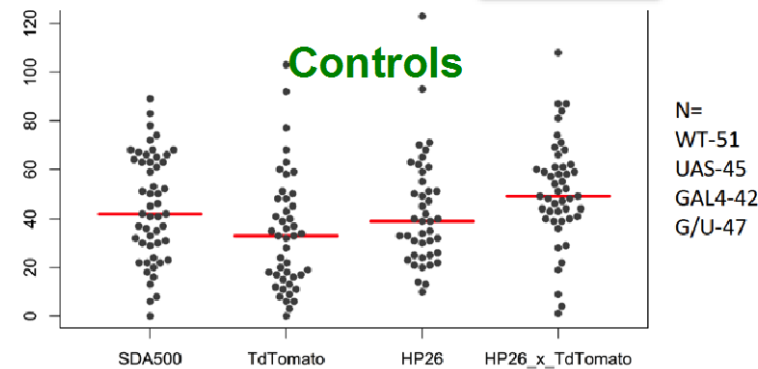
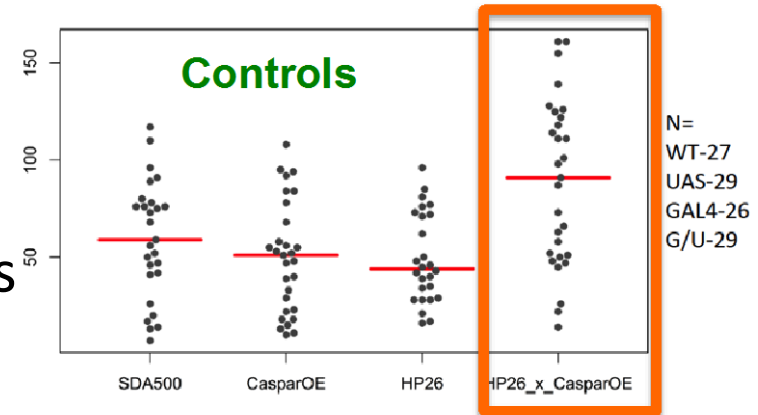
Genetically Modified Mosquitoes and a Malaria Vaccine



Create Immune-compromised Mosquitoes

Transposon-based Transgenics

Binary Gene Expression System

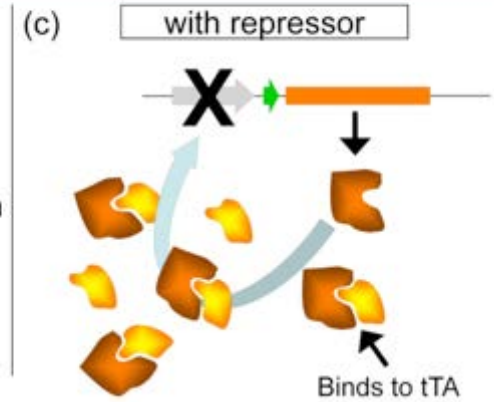
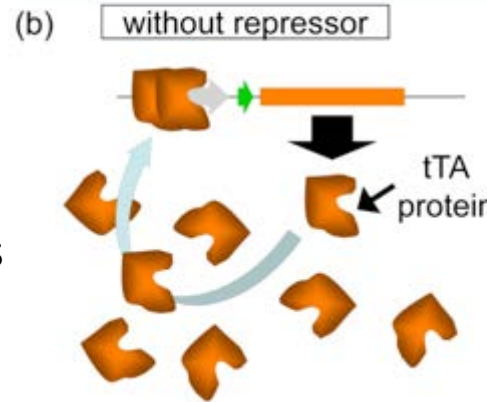
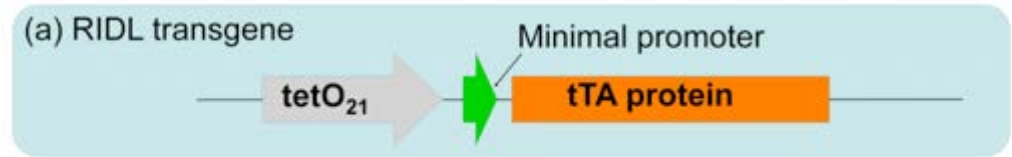
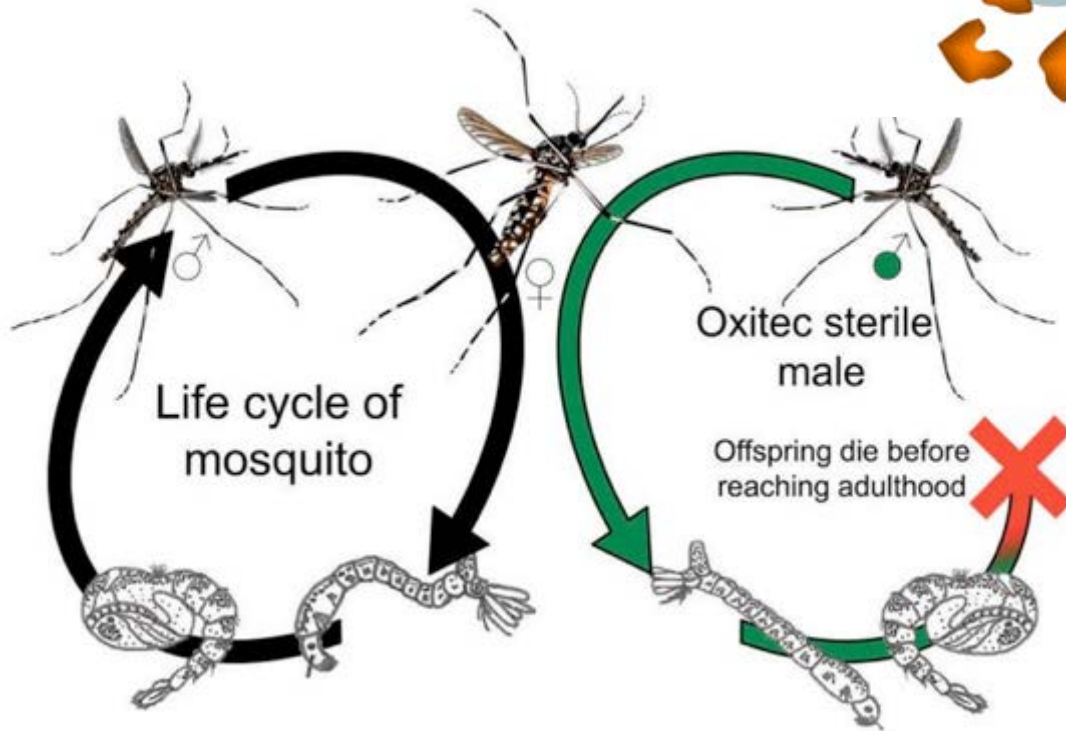


Genetically Contained Applications

Oxitec, LLC

RIDL Strategy

Release of Insects with Dominant Lethals



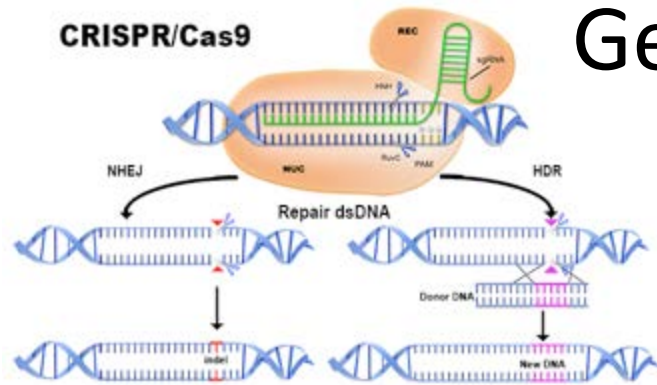
run-away transcription

Transposon-based Transgenics

Conditional Promoter

Uncontained Applications

Gene Drive Systems

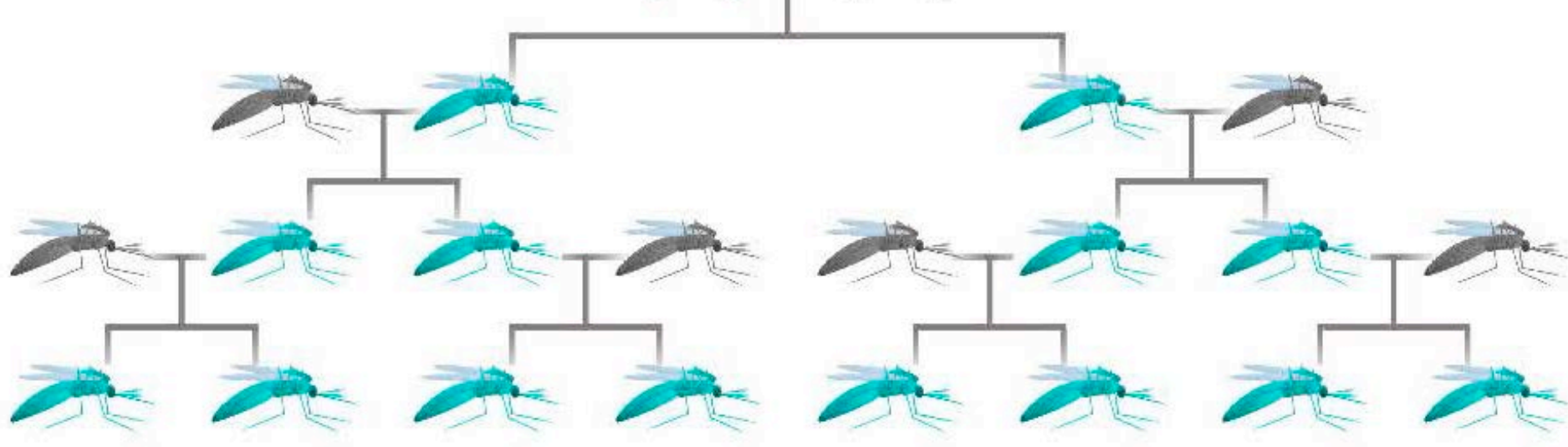


A

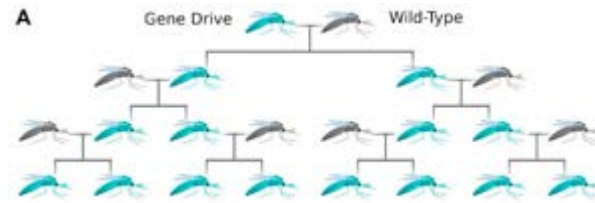
Gene Drive



Wild-Type



Gene Drive Systems



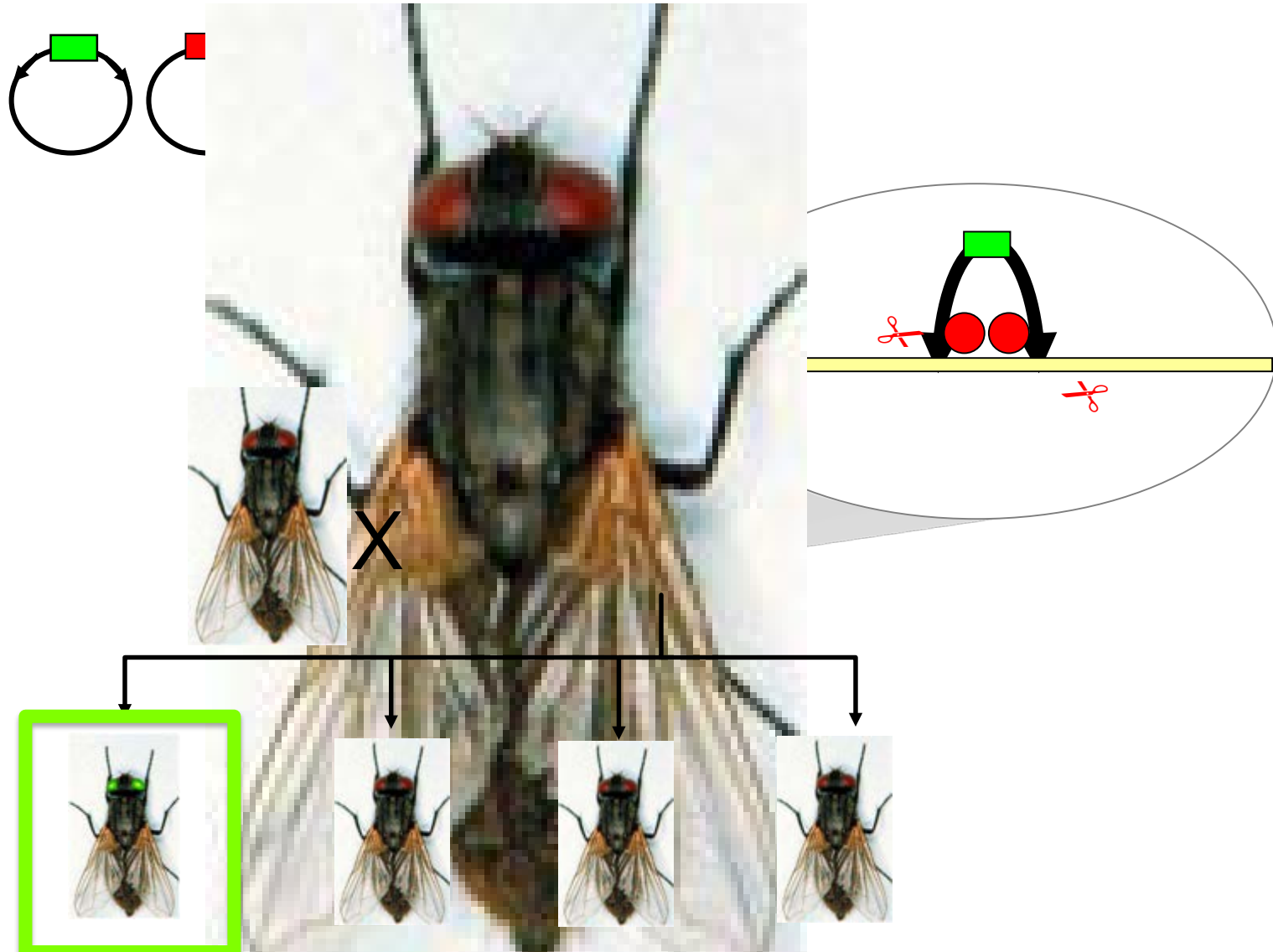
Erradication:

Sex-Ratio Distortion
Reduce Fitness

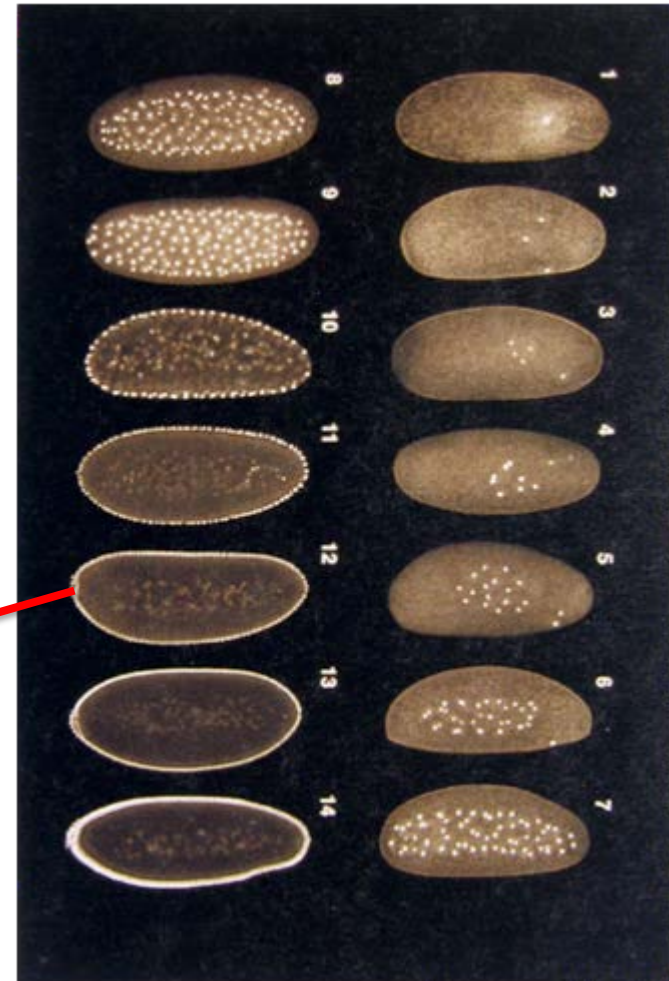
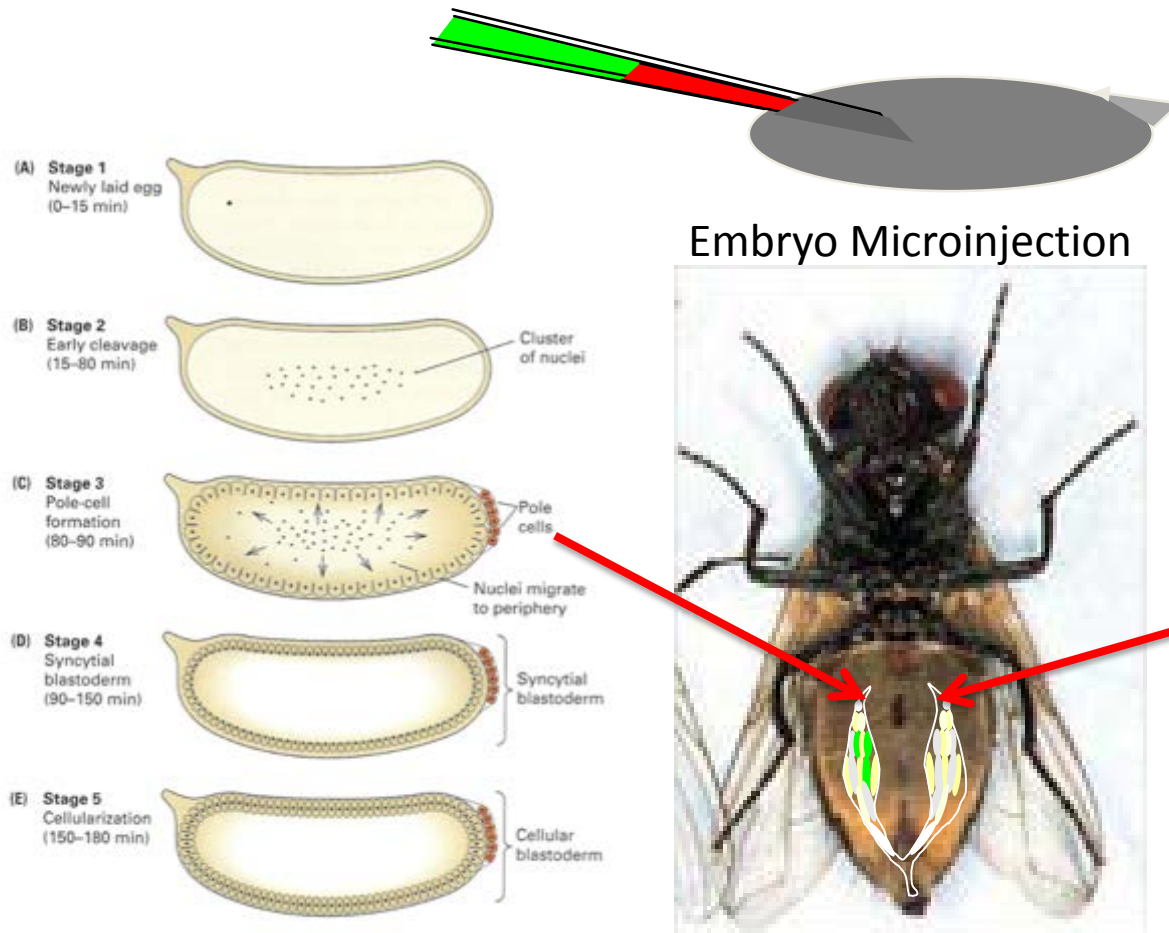
Gene Introgression:

Anti-parasite genes
Anti-virus genes

General Strategy for Genetically Modifying Insects



Delivery of Genetic Technologies to Insect Germ Cells



Aedes aegypti



Delivery by Early-Embryo Microinjection

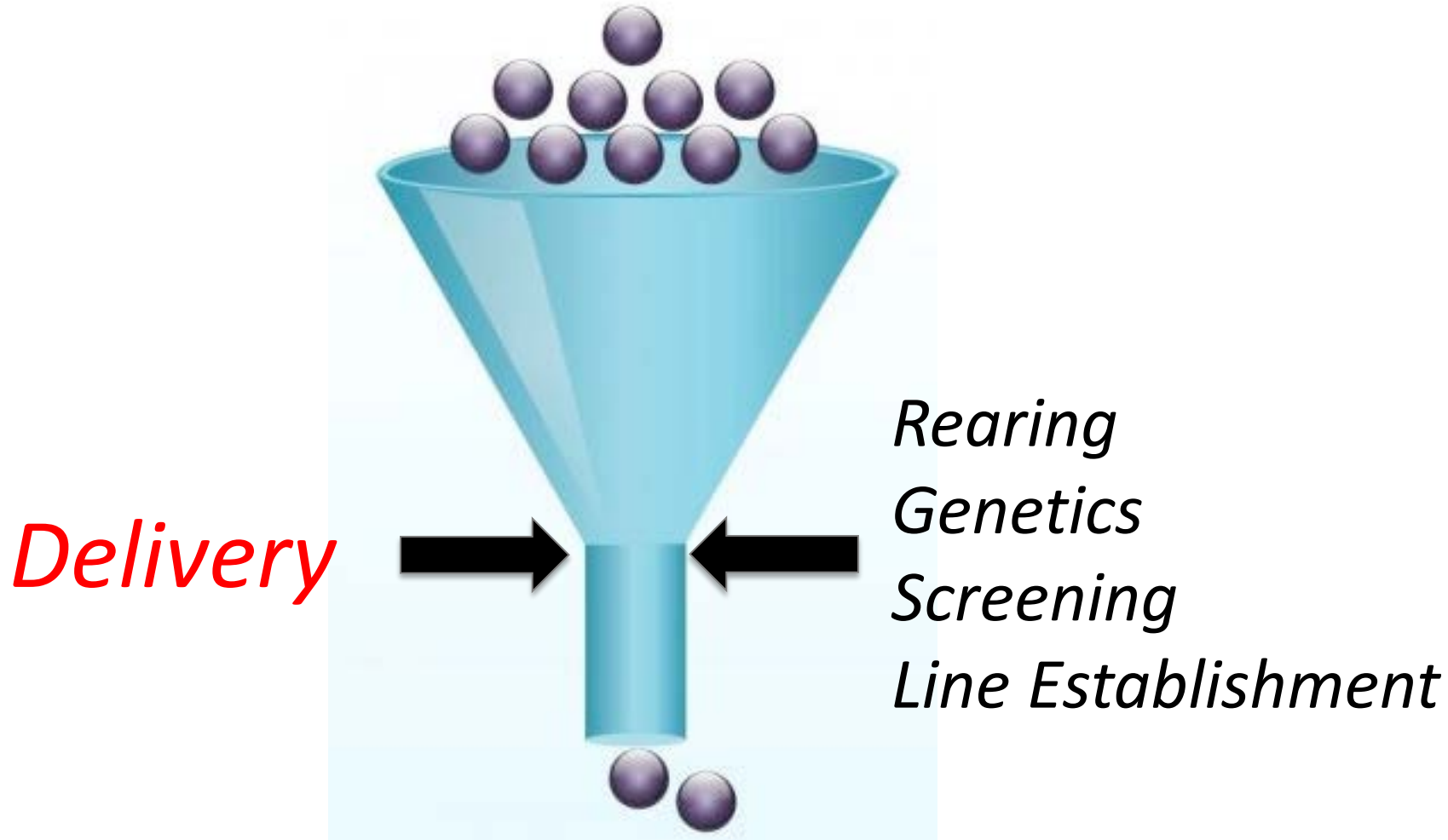
Limits Availability of Technologies



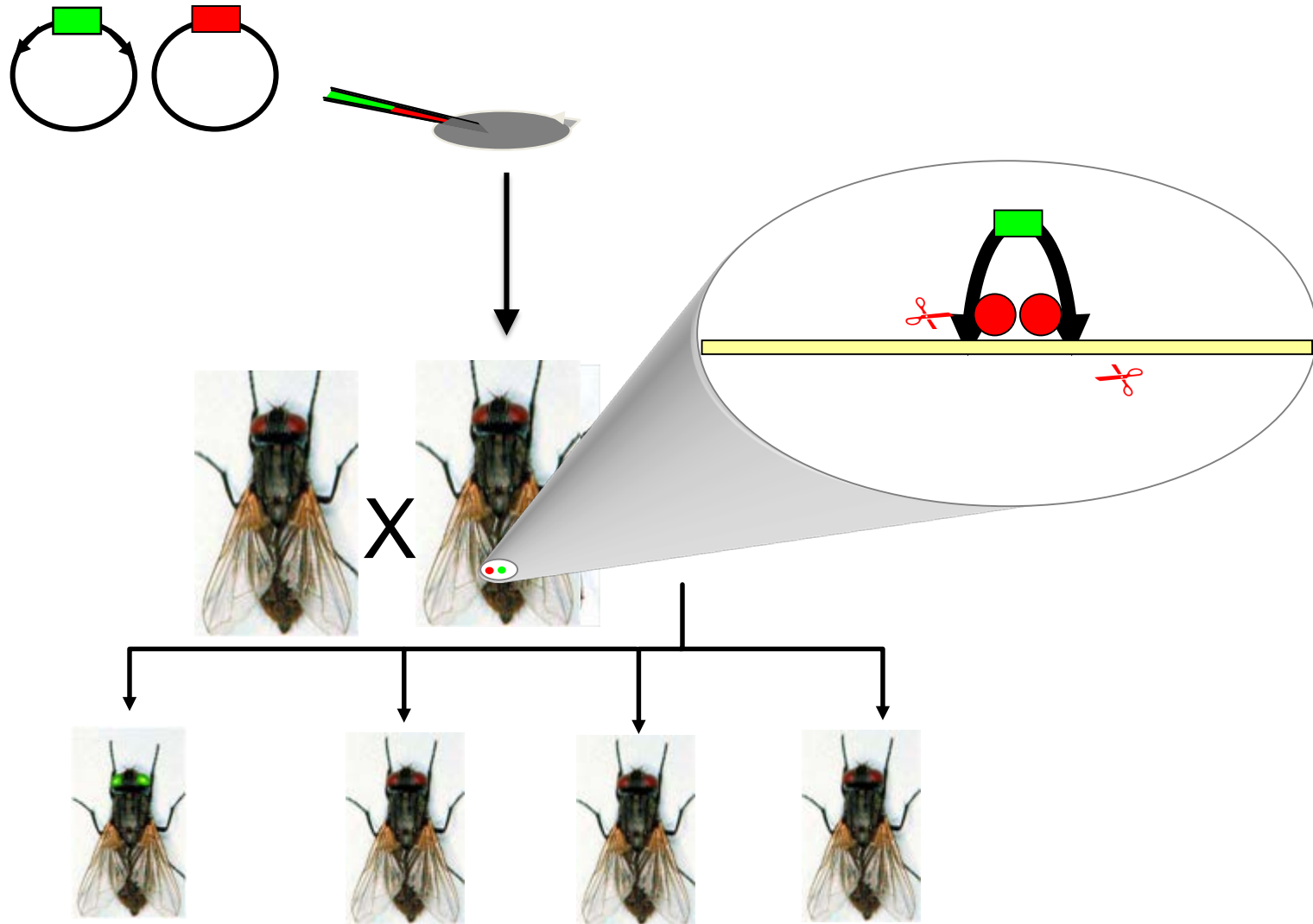
Early-Embryos Inaccessible in Many Species



Technical Challenges Limiting the Use of Insect Genetic Technologies



General Strategy for Genetically Modifying Insects

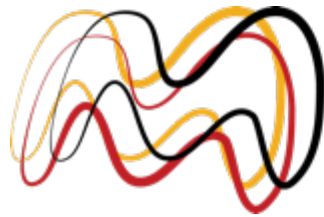


For Most Insect Scientists Insect Genetic Modification is a Heavy Lift



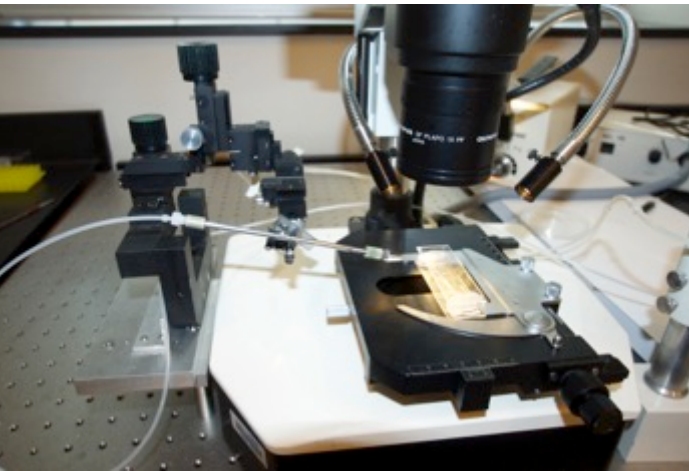


The Insect Transformation Facility

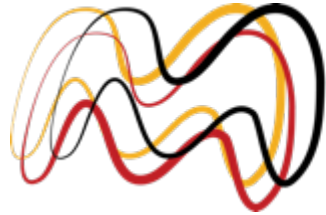


UNIVERSITY OF MARYLAND | NIST
INSTITUTE FOR BIOSCIENCE
& BIOTECHNOLOGY RESEARCH

Since 2006



The Insect Transformation Facility



UNIVERSITY OF MARYLAND | NIST
INSTITUTE FOR BIOSCIENCE
& BIOTECHNOLOGY RESEARCH
Since 2006

Insect Genetic Modification Services:

Transposon-based technologies

Site specific recombination technologies

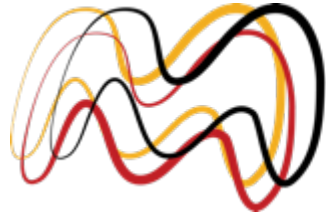
Endonuclease-based 'gene editing'

Training

Consulting



The Insect Transformation Facility

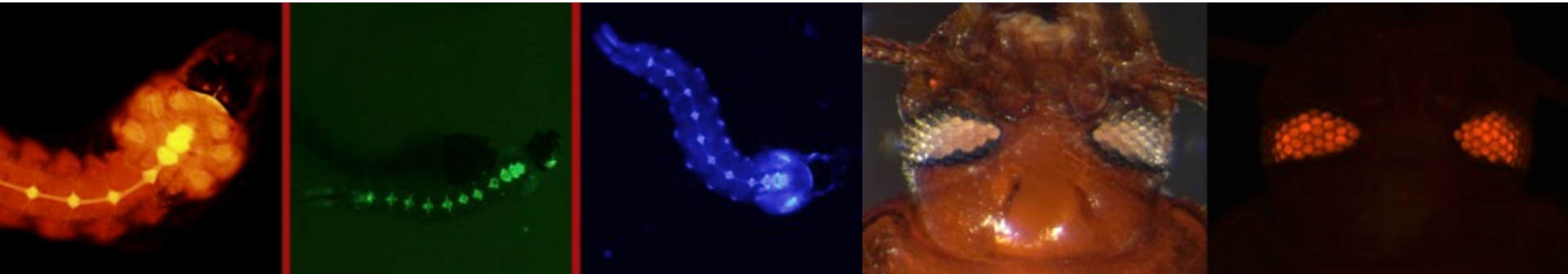


UNIVERSITY OF MARYLAND | NIST
INSTITUTE FOR BIOSCIENCE
& BIOTECHNOLOGY RESEARCH
Since 2006

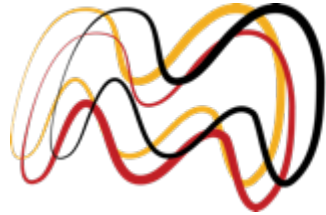
400+ Projects

55 Projects in FY2017

40+ Species



The Insect Transformation Facility



UNIVERSITY OF MARYLAND | NIST
INSTITUTE FOR BIOSCIENCE
& BIOTECHNOLOGY RESEARCH
Since 2006

Common Species:

Aedes aegypti

Anopheles stephens

Anopheles gambiae

Tribolium castaneum

Drosophila virilis

Drosophila simulans

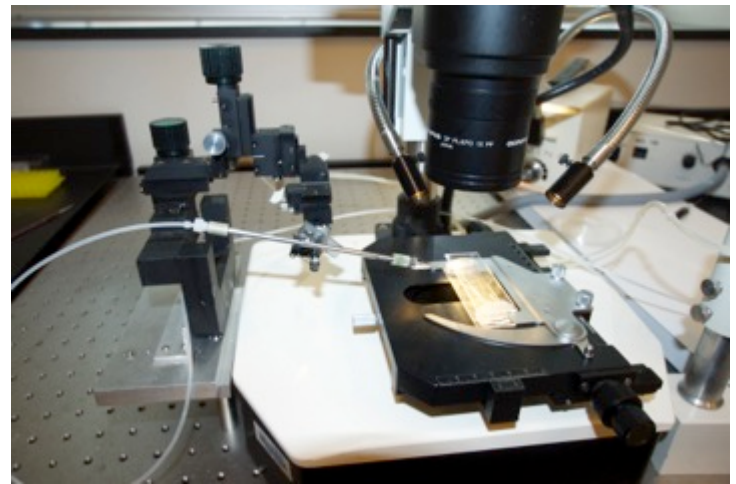


Common Technologies:

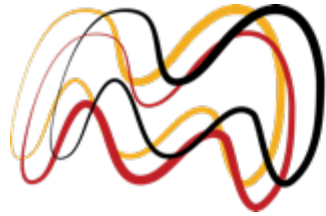
Transposons

Site-specific Recombination

CRISPR/Cas



The Insect Transformation Facility



UNIVERSITY OF MARYLAND | NIST
INSTITUTE FOR BIOSCIENCE
& BIOTECHNOLOGY RESEARCH
Since 2006

Recent Species:

Bemisia tabaci

Phlebotomous papatasi

Anopheles arabiensis

Anopheles funestis

Hermetia illucens

Ixodes scapularis

Sitobion avenae

Rhopalosiphum padi

Gryllus bimaculatus

Challenges:

What's Regulated?

Containment requirements?

Shipping requirements?

Insect Genetic Technologies

Why, How

Biosafety Challenges

David O'Brochta, Ph.D.

Institute for Bioscience and Biotechnology Research

Department of Entomology

University of Maryland, College Park

dobrocht@umd.edu

