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INSTITUTE FOR BIOSCIENCE
& BIOTECHNOLOGY RESEARCH

Creation and Maintenance of Transgenic Insects: From practical point of view

Transgenic Insects:
Micro-manipulation and Micro-injection of Disease Vectors

Channa Aluvihare
Insect Transformation Facility
Institute for Bioscience and Biotechnology Research
University of Maryland - College Park
9600 Gudelsky Drive
Rockville, MD 20850-3467
USA

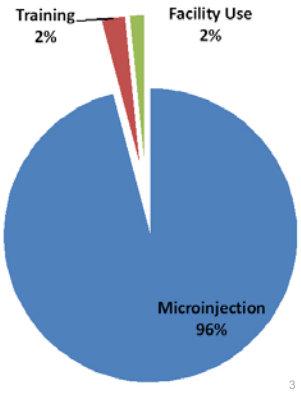
Transposable Elements for Insect Transformation ☆

AM Handler, United States Department of Agriculture, Gainesville, FL, United States
DA O'Brochta, University of Maryland, College Park, MD, United States

Insect Transformation Facility (ITF)

Core Facility - a discrete unit within an institution -dedicated personnel – equipment - space for operations.
recover cost providing service in the form of user fees that are charged to an investigator's funds, often to NIH or other federal grants.

The ITF mission is to be an international resource for the creation of genetically modified insects, a developer of new insect genetic modification technologies, and a source of training in the use of these technologies.



Client Research Focus

- **Host Pathogen Interactions (Malaria, Zika, and Dengue)**
- **Mosquito Olfaction**
- **Sterile Insect Technology**
- **EVO-DEVO**
- **Transposon Research**
- **Improvement of Beneficial Insects**
- **Gene Drive**
- **Food Security**
- **Malaria Vaccine Development (Sanaria Inc)**

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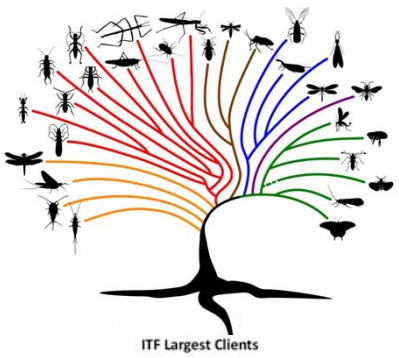
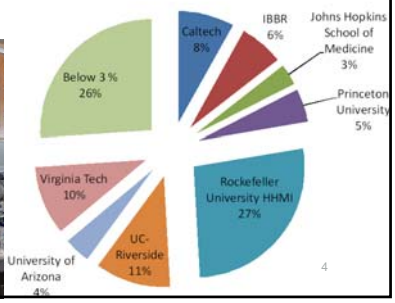
ITF Staff have experience working with 41 arthropod species:
Hermobia domestica, Lucilia sericata, Lucilia cuprina, Apis mellifera, Nezara viridula, Spodoptera frugiperda, Helicoverpa zea, Heliothis virescens, Cydia pomonella, Ixodes scapularis, Musca domestica, Macrosteles fascifrons, Toxotrypana curvicauda, Megaselia abdita, Borbotillus frigipennis, Culex pipiens, Culex tarsalis, Teleopsis dalmanni. Bemisia tabaci, Culex tarsalis, Cerapachys biroi, Lutzomyia longipennis, Nasonia vitripennis, Polistes carolina, Wasps sp (?), Vanessa sp.


ITF Largest Clients

- Clients in 8 countries
- Projects or submitted grants with 6 companies
- 28 Universities, Colleges, and Research Institutes
- 3 US government entities;
- 16 client publications since 2011.



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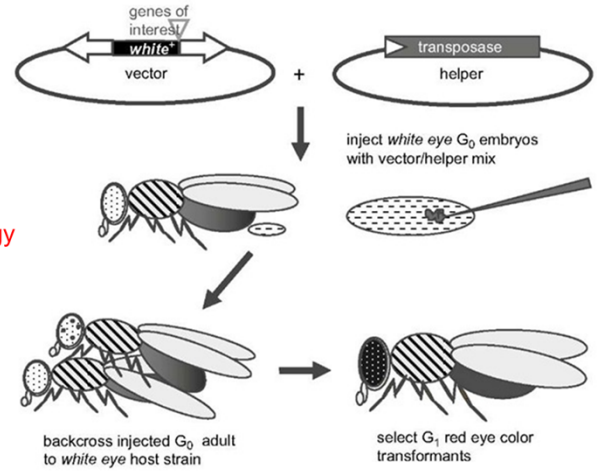
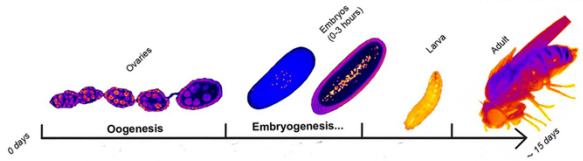
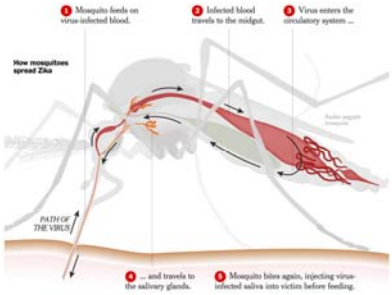
   - Embryo Preparation
   - Needles
   - DNA Preparation and Injection
   - DNA injection
   - Post-Injection Treatment
   - Improvements for Transformation Methodology



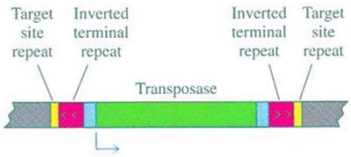


Some concepts

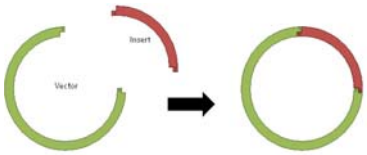
- Disease vector

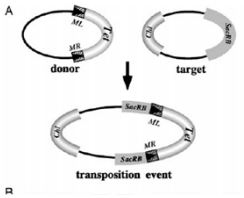


- Gene Vector

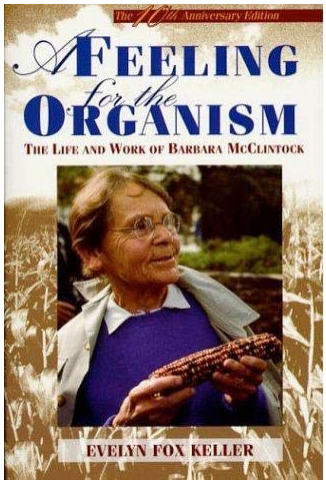


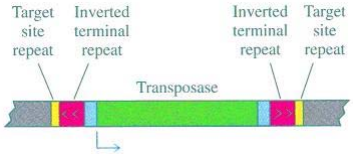
- Plasmid vector

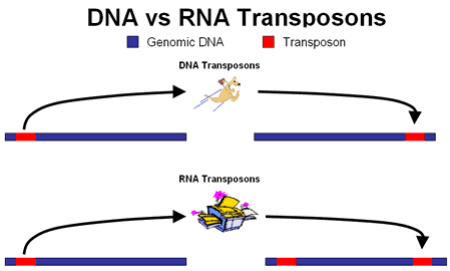




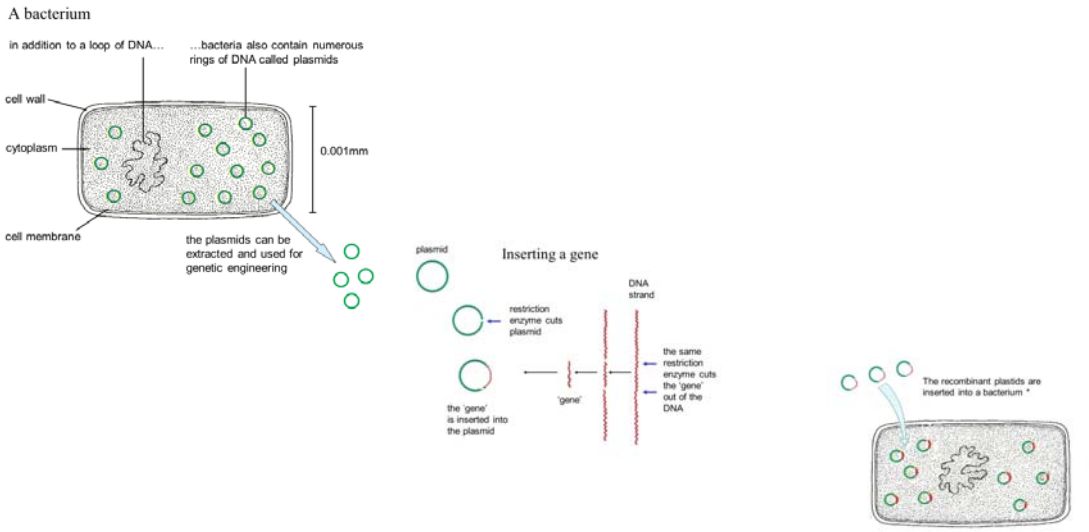
Gene Vector



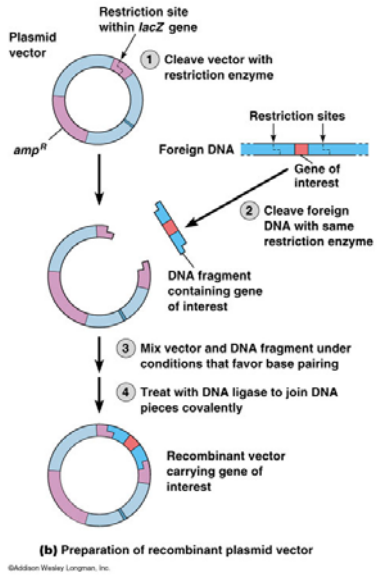




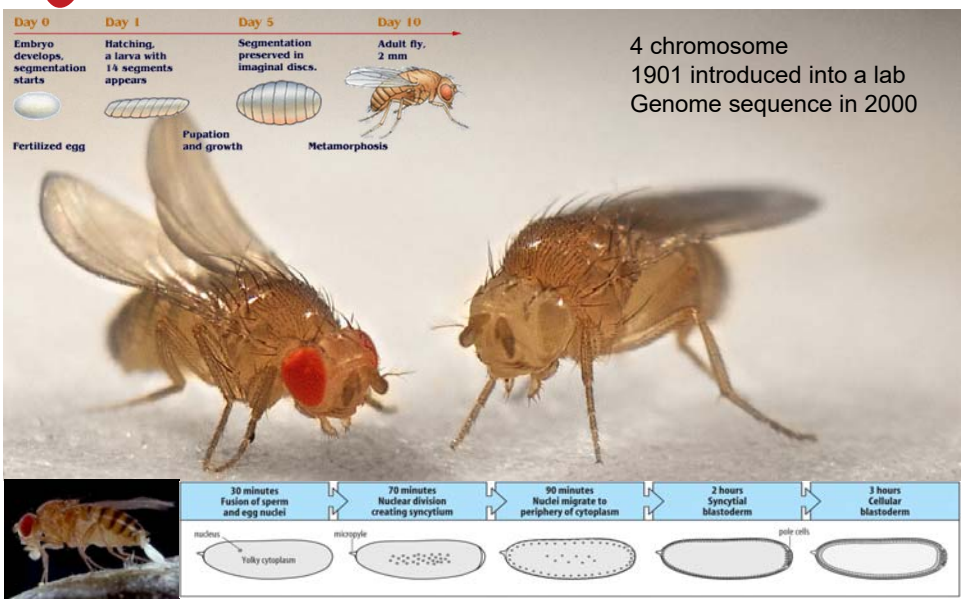
Plasmid Vector



Constructing a plasmid vector



Drosophila melanogaster Scientific work horse



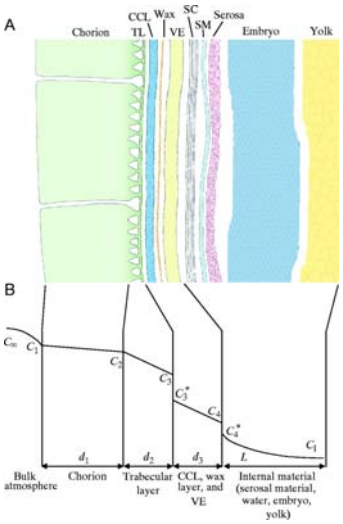
4 chromosome
1901 introduced into a lab
Genome sequence in 2000

(A) Schematic of an insect eggshell, drawn from published information on Manduca and Drosophila.

chorion,
trabecular layer (TL),
crystalline chorionic layer (CCL),
wax layer,
vitelline envelope (VE),

In place at oviposition

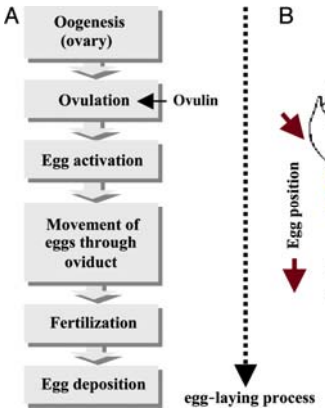
serosal cuticle (SC),
serosal membrane (SM),
serosa,
embryo,
yolk



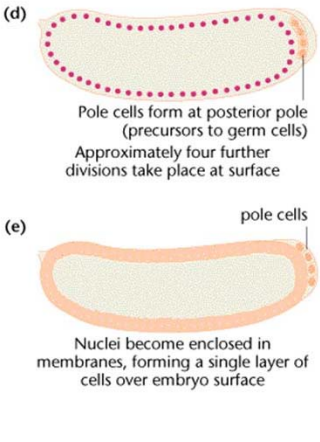
H. Arthur Woods et al. J Exp Biol 2005;208:1297-1308

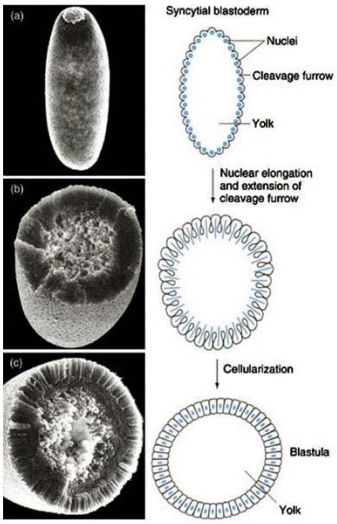
Journal of Experimental Biology

Early development in *Drosophila*



After thirteen divisions membranes ingress from the cortex to enclose each nucleus, to form the *cellular blastoderm*.





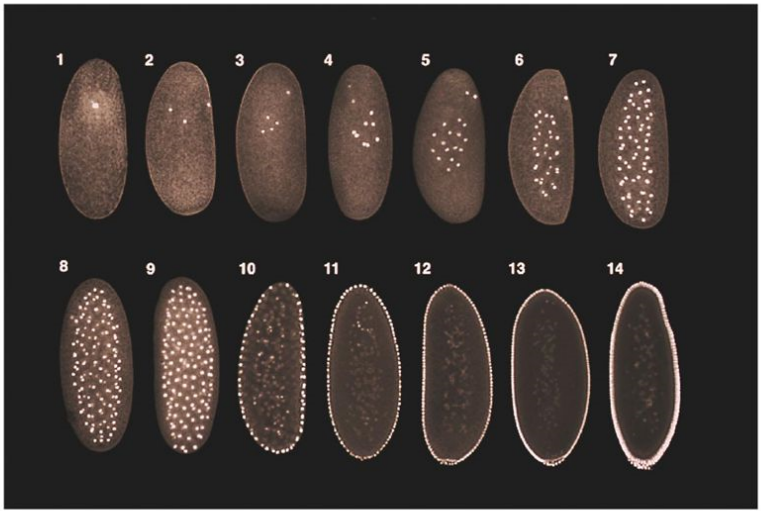
Superficial Cleavage in a *Drosophila* early Embryo

Syncytial blastoderm

Numbers refer to the cell cycle

When to inject ?

1
2
3
4
5
6
7
8
9
10 – 512 nucleus pole cells form
11
12
13
14

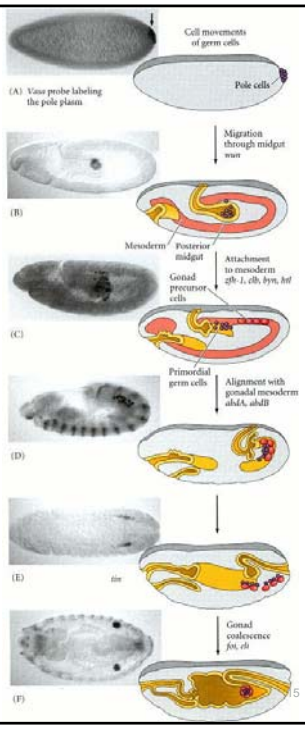


DEVELOPMENTAL BIOLOGY, Seventh Edition, Figure 9.1 Sinauer Associates, Inc. © 2003 All rights reserved.

Laser confocal images of stained chromatin courtesy of W. Baker and G. Schubiger

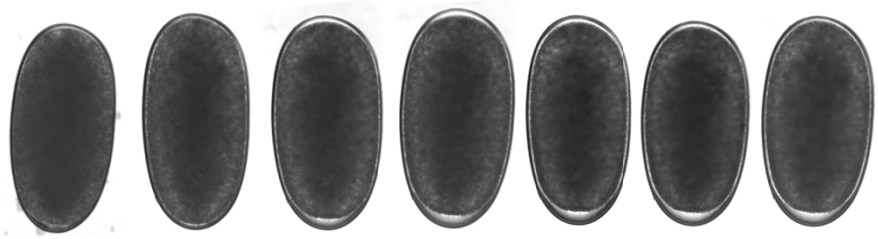
Localization of the cytoskeleton around nuclei in the syncytial blastoderm of Drosophila

Migration of germ cells in Drosophila embryos

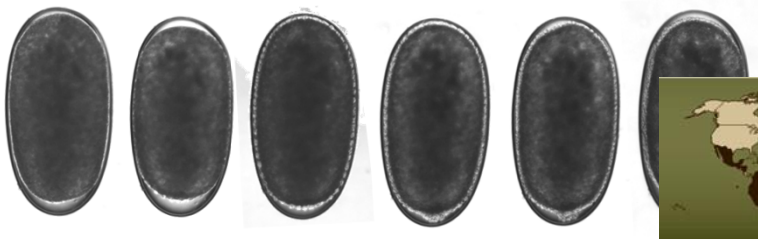


From Karr and Alberts 1986; photographs courtesy of T. L. Karr

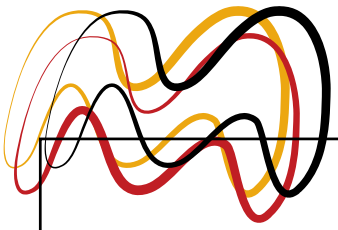
Coffee Berry Borer



0 a 2 h | 2 h | 3 h | 3 h 30m | 4 h 30m | 5 h 30m | 6 h 30m



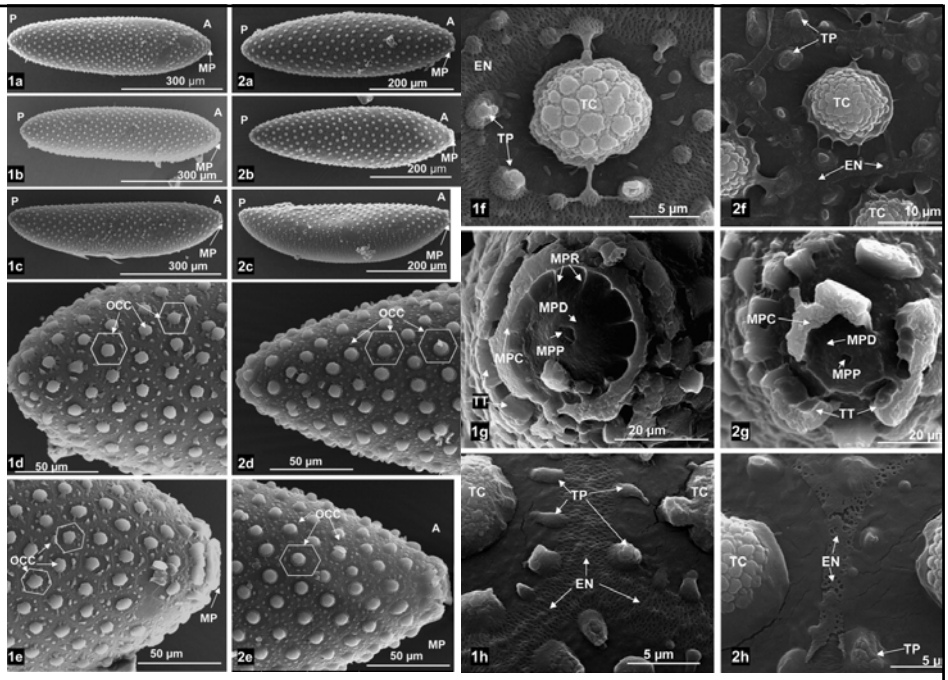
7 h 30m | 8 h 30m | 9 h 30m | 10 h 30m | 11 h 30m



Aedes aegypti 1 a- h

Aedes albopictus 2 a-h

P- Posterior end,
A- Anterior end,
MP- Micropylar apparatus,
OCC – Outer Chorionic Cell,
EN – Exochorionic Network,
MPC – Micropylar Disc,
MPP – Micropylar Pore,
MPR – Micropylar Ridge,
TC – Centreal Tubercle,
TP – Peripheral Tubercle,
TC – Tooth-like Tubercle



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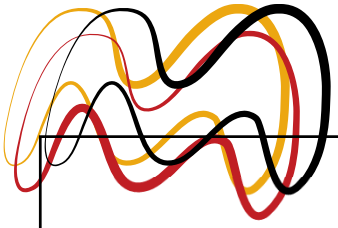

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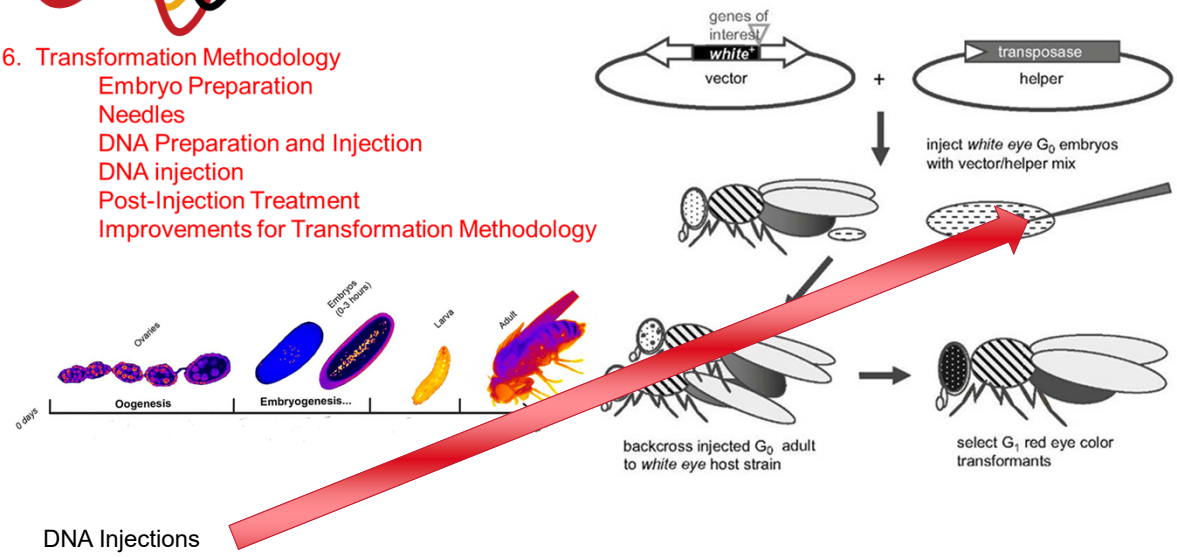

DNA Injections

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Manufacturing Quartz Capillary Needles by Sutter P2000 Laser Puller

Use 7.5 cm long 1.0 mm OD & 0.7 mm ID Capillary

Can make two needles with one Capillary

Needles are fused at the tip

Genetic materials are placed in the needle using a small glass Micro-pipette

Once the needle is mounted on the micro-manipulator it needs to be open physically

Changing parameters for heat, filament scanning, velocity, delay in applying pull force we can design needles appropriate for varying egg chorion

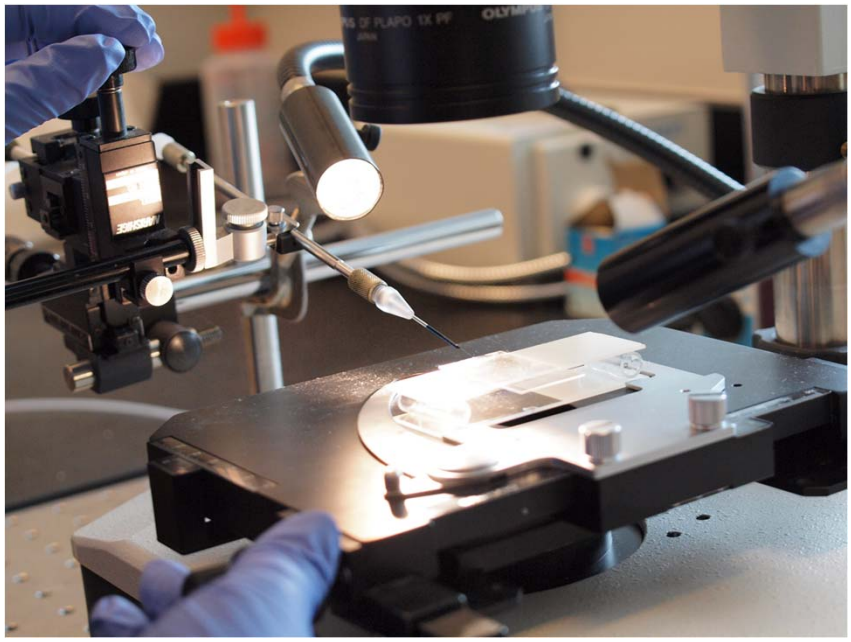
Typical Micro-injection Setup

Needle and needle holder are attached to micro-injection apparatus (not shown)

Above is attached to X,Y,Z micro-Manipulator on a metal stem

Micro-manipulated eggs are on glass cover slide

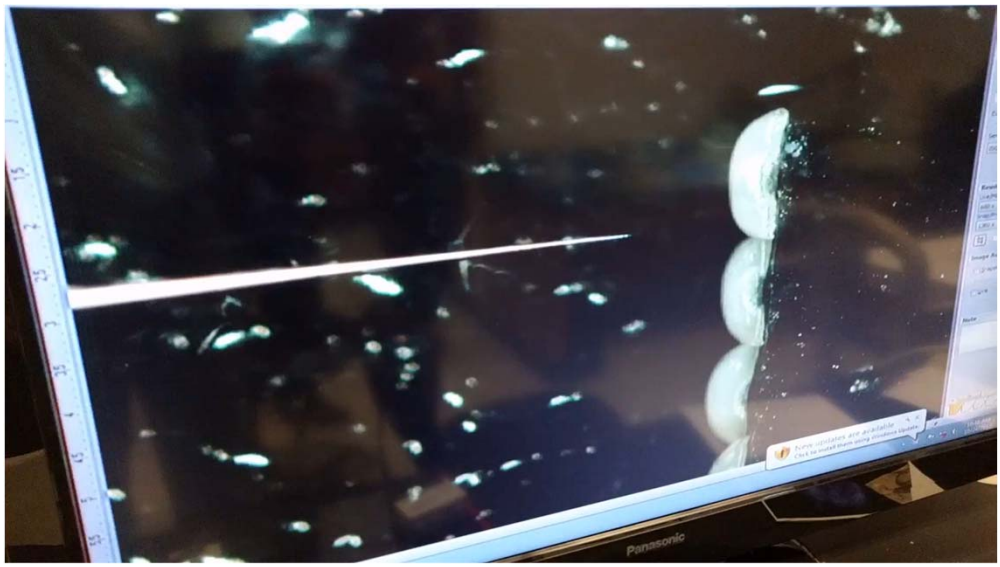
Injections are performed by moving the into each egg for injections



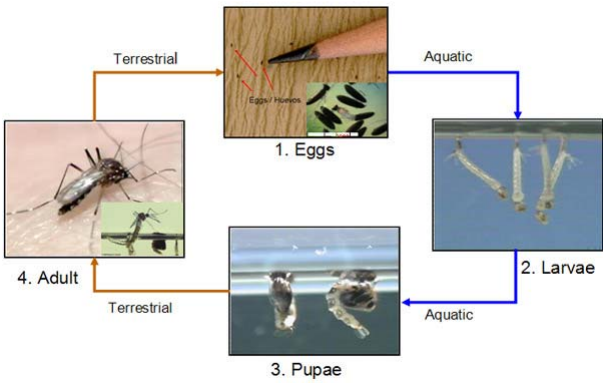
Creating Insect Genetic Modifications

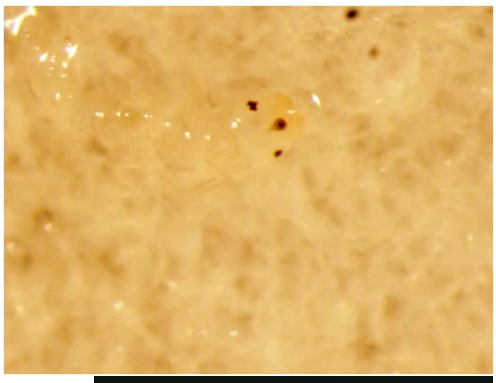
Aedes aegypti Microinjection posterior

Anopheles stephensi egg injections ventral

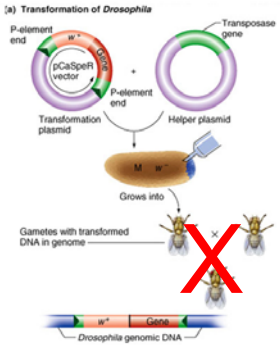


Post micro-injected eggs





Transformation of Drosophila



Mosquitoes Cross Ratio
♂ : ♀ 1 : 7

Typical Project
Micro-inject = 500 eggs
G0 = 20 % hatch = 100
Crosses= 10 ♂ & 5 ♀
G1 = 52,500 + 7,500

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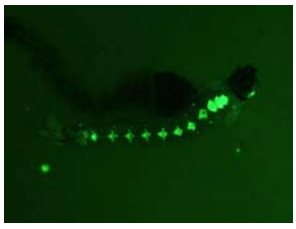
Screening G1



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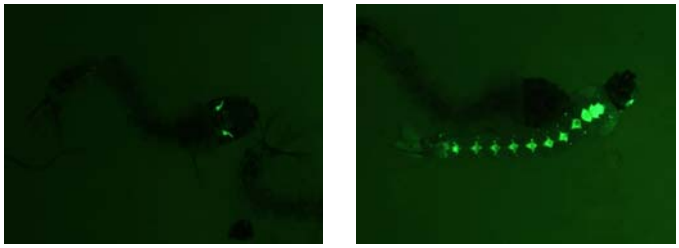
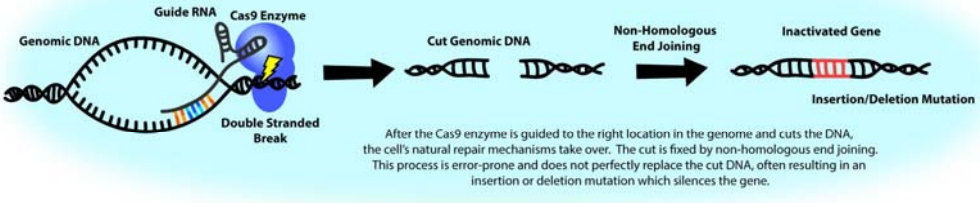
Genome Modification Transposons

- Limitations
  1. Insertion is basically random
  2. Subject to position effects



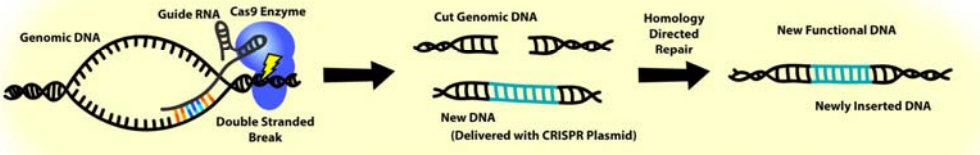
3. Need promotors that work within the insect of interest in order for the system to work
4. Do not work equally in all insects
5. Can be remobilized

Gene Silencing with CRISPR



After the Cas9 enzyme is guided to the right location in the genome and cuts the DNA, the cell's natural repair mechanisms take over. The cut is fixed by non-homologous end joining. This process is error-prone and does not perfectly replace the cut DNA, often resulting in an insertion or deletion mutation which silences the gene.

Gene Insertion with CRISPR



To insert a gene, the new gene is added into the original CRISPR plasmid. It is designed to line up perfectly with the cut DNA strands, so the cell uses a different technique, homology directed repair, to incorporate a new stretch of DNA into the genome.

Thank you!

Insect Transformation Facility Team

Professor David O'Brochta
Rob Harrell – Manager
Yonas Gebremicale
Robert Alford
Valeria Saffer

IGTRCN Workshop Participants
July 2017