Class II: Manipulation of the *Drosophila* genome

P element mediated germline transformation
- enhancer and gene traps
- GAL4/UAS system
- FLP/FRT system
- phiC31 system
How can you use P elements for transgenesis? (get DNA in, know it’s in, and track it)

- w+/w- any DNA
- Plasmid
- P[w+]
- Wings clipped
- F1 Progeny
- transposase
- Functional LTRs
- 0 1 2 3
Manipulation of fly genome for discovery

1) Genetic control of transposition
   A) Chromosomally stable source of transposase: CyO [Δ2-3]
   B) X chromosome with one or more P[w+] elements

Control Cross:

- w- P[w+]/Y X w-/w-
- w- P[w+]/w-
- w-/Y

All females: orange-eyed
All males: white-eyed

- w- P[w+]/Y; CyO [Δ2-3]/+
  X w-/w-
- w- P[w+]/w-?
- w-/Y?

Most females: orange-eyed, but some white-eyed
Most males: white-eyed, but some orange-eyed
w- P[w+]/Y; CyO [Δ2-3]/+
Manipulation of fly genome for discovery

1) Genetic control of transposition
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Control Cross:
w- P[w+]/Y \times w-/w-
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Most males: white-eyed, but some orange-eyed
PART II: Enhancer traps, gene traps, GAL4/UAS system
31 Flavors of P elements

1) Insertional mutagen:

2) Enhancer Trap:

- lacZ, tau-LacZ, GFP, etc
31 Flavors of P elements

3) Gene Trap:

Lynn Cooley: Yale
flytrap.med.yale.edu/

Morin X et al. PNAS 2001;98:15050-15055
4) Gal4-UAS System (Brand and Perrimon, 1993)

Yeast:
- GAL4
- UAS
- DNA
- GAL4-inducible genes

Flies:
- Basal promoter
- Characterized enhancer
- Any gene
- GAL4
dpp-GAL4

UAS-eyeless
dpp-GAL4 drives gene expression down the middle of each imaginal disc.

Eyeless encodes the Pax6 transcription factor and is necessary for eye development in flies (and mice and humans).

What happens when we drive eyeless in all leg imaginal discs?
Split-GAL4 and GAL80

Gene expression =
Other inducible gene expression systems

LexA-Operator

QUAS (Neurospora)

Persad et al. Journal of Plant Science, 2020
Part III: FLP/FRT System
FLP-FRT system (yeast)

A) FLP recombinase: Site-specific recombinase

B) FLP Recombinase Target sites (FRT) – 34 bp minimal site

C) Consequence of FLP-mediated recombination depends on the orientation of FRT sites on DNA

1. Direct repeats – same DNA strand

   [Diagram showing 1 2 3 + FLP 1 2 3 Resolution]

2. Inverted repeats – same DNA strand

   [Diagram showing 1 2 3 4 + FLP 1 3 2 4 Resolution]

3. Direct repeats – different DNA strands

   [Diagram showing 1 2 A + FLP 1 2 B and 1 2 m + FLP 1 2 m Mitotic Recombination]

   [Diagram showing 1 2 m + FLP 1 2 m and 2 + + 2 + +]
Creation of molecularly defined deletions

1) Generate huge libraries of the following two types of P elements:

2) Place nearby P elements of each type in trans to each other and add FLP.
Part IV: phiC31 integrase system
**phic31 integrase system**

A) phiC31 integrase catalyzes site-specific recombination between **attb** and **attp** attachment sites

B) Uses – phiC31 landing pads: place **attp** site within P element and creates scores of random insertions in each chromosome. Then one can insert any DNA in the same site (removes issue with position effect).

C) phiC31-based libraries

1. RNAi libraries:

2. ORFeome:

3. Regulatory regions:
Drosophila gene disruption project

Goal: For every gene, assess its loss of function phenotype, gene expression pattern, subcellular localization, interacting proteins, and rescue its mutant phenotype.

MiMIG: Minos-mediated integration cassette

Create and map thousands of random MiMIG insertions

Identify those in coding introns

Use recombinase-mediated cassette exchange to swap out different cassettes to assess gene function
Protein trap orientation

Gene trap orientation

+ phiC31 integrase

Protein trap allele

Gene trap allele

OR