Transgenesis: Insert exogenous DNA into the genome

Requirements for transgenesis:

1. DNA must insert into the genome.
2. You need to know it got in—i.e., you need to be able to track the DNA across generations.
3. Exogenous DNA must stay in the genome.

Potential of Transgenesis—what can you do with it:

Go over: 1) Key aspects of transgenesis

A) Transposons
   - P elements, PiggyBac, Iac-Z, MNPV

B) Power of a transgenic toolbox—a fly's eye view: think about how to use technology to other systems using CRISPR.

P elements: DNA transposon; jumps as a DNA intermediate

1) Autonomous P elements:
   - Can jump on own
   - 2907 bp

2) Non-autonomous P element:
   1. Intact LTRs = has potential to jump
   2. Internally deleted = Non-functional

   - Can't jump on its own
How can you use P elements for transgenesis?

1. P element mediated transposition: separate LTRs + ORFs

- Keep LTRs
- Pitch LTRs

2. ANY DNA

3. Fusion LTRs

4. ANY DNA

5. White gene

6. Marked

7. 3xP3 ds REP

8. GFP

9. w+/w-

10. w-/w-

11. P

12. GAL4

13. Pole cells (germline)

14. Co-infect

15. w-/+; w- adult

16. w-/w-

17. w-/+; w- adult

18. w-/+ or w-/+; w- adult

19. w-/+; w- adult

20. Genotypically mutant for white gene

21. But if P[C[w+]] inserted into genome ⇒ eyes are yellow, orange, red.

22. P[C[w+]] = stable + can follow
Can get DNA in, it's stable, and you can track it.

What can you do w/ it? What are limitations?

1) Genetic control of Transposition
   * a) Chromosomally stable source of transposase: Cyo[Δ2-3]
       → 23 P element transposase
       Piggyback LTRs
   * b) X-chromosome w/ one or P[w+] elements

Control Cross Scheme: \[ \frac{W-}{W-} \times \frac{P[w^+]}{W-} \]

\[ \begin{align*}
   &W- \\
   \downarrow \\
   &W- \\
   \downarrow \\
   &W- \\
   \downarrow \\
   &W- \\
   \downarrow \\
   &W- \\
   \downarrow \\
   &W- \\
   \downarrow \\
   &W- \\
   \downarrow \\
   &W-
\end{align*} \]

All \( \varphi \) = orange-eyed

All \( \sigma^2 \) = white-eyed

Expt Cross Scheme: \[ \frac{W-}{W-} \times \frac{P[w^+]}{Cyo[Δ2-3]} \]

\[ \begin{align*}
   &W- \\
   \downarrow \\
   &W- \\
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   &W- \\
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   &W- \\
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   &W- \\
   \downarrow \\
   &W- \\
   \downarrow \\
   &W-
\end{align*} \]

Most orange-eyed

but some white-eyed

Most white-eyed

but some orange-eyed

1) Can mobilize P element to jump from one chrom to another
   \[ 6 \times P[w^+]; [Δ2-3] Cyo \]

2) Why important?
   - can put any DNA w/ in P element (≤ 40kb)
   - can jump anywhere in genome (now w/ CRISPR scan target)
Uses or flavors of P elements

1) Insertional mutagen
   - Can insert in gene + disrupt function
   - Tns insert randomly
   - Collection of Tns

2) Enhancer Trap:
   - Identify genes based on their expression pattern

3) Gene Trap

4) Gal4-UAS System (Brand-Perrimon '93)
   A) Yeast GAL4-UAS system:
   B) Fly GAL4-UAS system

  Drive any gene in any pattern - gene rescue, gene misexpression

5) Split-GAL4 System: Intersectional Gal4:
   - GAL4 + Gal80 DNA binding
   - split GAL4

- PC111
- Tpa libraries (Pbocc mouse library)
- Enhancer
- $\beta$ +
- basal promoter
- exon 1
- exon 2
- GAL4 driver
- UAS-linked responder
- UAS
- 1A
5) FLP-FLP system

A) FLP recombinase: Site-specific recombinase (yeast)

B) FLP Recombinase Target sites: 34 bp minimal site

C) Consequences of FLP-mediated recomb depends on orientation of FLP sites

1. Direct repeats same molecule:

   1. Inverted repeats

   2. Direct repeat diff molecules

   3. Metotic recomb:

D) FLP-out Gal4

   ActS5 → Gal4 → Yorkie
6) Phi C31 Integrate system:

A) Phi C31 integrate catalyzes site-specific recombination between attB and attP attachment sites.

Advantages of Phi C31 system:
1. Can insert any piece of DNA into attB site.
2. Phi C31 landing pad:
   1. Place attB site within P element
   2. Generate scores of random insertions in each chromosome
3. Removes issue of position effect — can insert many genes in same site
4. Phi C31-based libraries:
   A. RNAi libraries
   B. ORFeome
   C. Regulatory regions

\[ \text{attB} \rightarrow \text{attB} \rightarrow \text{attB} \rightarrow \text{attB} \times 1000s \]
For every gene, assess the following:
1) Loss of fcr-10 phenotype
2) Gene expression pattern
3) Subcellular localization
4) Identify associated proteins
5) Rescue of induced mutant phenotypes.

- MiMCL - Minos-mediated integration cassette

\[
\text{Minos} \downarrow \text{atf} \quad \text{ΔSTOP:EGFP-PolyA-yellow} \quad \text{atf} \quad \text{Minos-P} \\
\downarrow \quad \text{Generate 716,000 lines} \quad \downarrow \quad \text{Identify those in coding introns}
\]

\[
\text{Minos} \downarrow \text{atf} \quad \text{ΔSTOP:EGFP-PolyA-yellow} \quad \text{atf} \quad \text{Minos-P} \\
\downarrow \quad \text{Swappable cassettes} \quad \downarrow \quad \text{atf}
\]

- Cassette 1: gene expression
- Cassette 2: protein localization
- Protein interactors
- Tissue-specific removal of protein fcr-10