FLP-FRT mediated F1 Mosaic Screen

\[ \text{EMS} \xrightarrow{FRT40} yw \text{Cy-FLP} \xrightarrow{w+GFP} \text{Screen in this generation} \]

Key points:
1. Mosaic recombinants are restricted to the eye.
2. Essentially all cells undergo recombinants; 50% w^-GFP.
3. Screen by each chrom. arm.
4. Screen in F1 generation + recover the mutation on the affected fly.
   - Can screen thousands + thousands of flies.

Many groups used the above screen to identify mutations that affect 

Results:
1. 23 genes identified
2. 26 from C. elegans
   a. Most larger cells than twin-spot
   b. Many more cells
3. Four genes exhibited the same phenotype:
   a. More cell proliferation (t cells)
   b. Less cell death (t cells)
4. Four genes:
   a. Salvenor - 60kD w^-MOTIF protein
   b. Warts - 100kD NDR kinase, PKY mdk
   c. Hippo - 66kD STE20 family kinase
   d. Mobb/Mots - 2.19 novel proteins
How identify the gene that when mutated yields the observed phenotype: excess proliferation?

1) Salvador
   - 4 alleles
   - Fine-scale genetic mapping localized saa to a 20 kb region.
     1. 5 genes in the region.
     2. Sequenced all 5 genes in all 4 alleles.
     3. 4 of 5 genes: no A in any background.
     1 of 5 (A): premature stop codon in each background.

2) Hippo
   - 8 alleles
   - Fine-scale genetic mapping localized hpo to ~40 kb.
   - Sequenced all genes in the region in all alleles: in each allele.
   - RNAi of this gene rescued the hpo mutant phenotype when driven under the control of a ubiquitous Gal4 driver line:
     
     | Tobrin-Gal4 | hpo 
     | UAS-Gene X | hpo 

     Kinase Domain  | Actomyosin Domain  | Dimerized Domain |

3) Mots/MOhS: 2190 x Novel Protein

4) Wats: 1609 x Kinase

S-PPXY Motifs

PPXY motifs interact with WW domain
Identify all known tumor suppressor genes

Obtain mutant alleles of all fly orthologs

Assess genotype

**Known:** Merlin = fly ortholog of NF2 tumor suppressor
1. Member of 4.1 superfamily of adapter proteins.
2. Expanded is another member of 4.1 superfamily.
3. Merlin −/− has slight overgrowth genotype
4. Merlin known to act in a partially redundant manner
5. Expanded −/− = slight overgrowth genotype

What do you want to know?
- ex−, mer− + ex− mer− 2° mutant

How to create cells doubly-mutant for ex + mer?

**Genotype:**

- **YW mer**
- **YW+mer**
- **+**

**Results:** 1) Genotype identical to say, hsp...

**Conclusions:**
1) mer+ ex− act in a redundant manner
2) mer− cell prof
3) cell death
Genetic epistasis: How to order the action of genes?

1) Have six genes, two are kinases.
   - all mutate to the same phenotype
   - likely a signaling pathway.

How can you figure out the relative action of each different gene?

1) Create or identify gain of fcrx alleles vs. the opposite phenotype.

   Approach: over-express each gene and see if it leads to cell death.

   a) Generate UAS-linked transgenes of each gene.

      Phenotype
      UAS → merlin → Little/no effect
      UAS → expanded → cell death if cell prolif.
      UAS → hpo → pathway is hyperactivated
      UAS → wts → little/no effect
      UAS → mobs →
      UAS → sev →

2) Create double-mutants b/w gain of fcrx alleles of ex or hpo and loss of fcrx mutations in other genes.

   a. Logic: 1. The loss of fcrx phenotype of a gene is epistatic to the gain of fcrx phenotype of any gene upstream of it.
      → fcrx of upstream requires that of the downstream.
      $A^x \rightarrow B \rightarrow C \rightarrow D \rightarrow E$

      2. The gain of fcrx phenotype of a gene is epistatic to the loss of fcrx phenotype of any gene upstream of it.
      $A \rightarrow B \rightarrow C \rightarrow D^x \rightarrow E$
Examples of Epistasis Experiments.

1) Gain of fox allele of expanded (too little growth) plus loss of fox allele of hpo (too much growth). 

   \[ \text{Ex-FLP} \rightarrow \text{hpo} \rightarrow \text{cell lethal} \] 

   \[ \text{GMRGAL4} \rightarrow \text{UTS-Expanded} \]

   **A. Result:** overgrowth genotype identical to hpo
   - hpo loss of fox genotype is epistatic to ex genotype
   - ex requires the fox of hpo to mediate its effect

2) Gain of fox allele of hpo plus loss of fox allele of wts

   **A. Result:** overgrowth genotype identical to wts.
   - hpo \rightarrow wts
   - ex \rightarrow hpo \rightarrow wts

What is downstream of Warts? (Huang et al., 2005)

1) Identify proteins that interact w Wts via a yeast 2-hybrid.

   \[ \text{GAL4} \rightarrow \text{DNA binding domain} \rightarrow \text{Protein A} \rightarrow \text{WTS} \]

   \[ \text{GAL4} \rightarrow \text{Trans-activator domain} \rightarrow \text{UTS} \]

   \[ \text{GAL4} \rightarrow \text{Marker/selectable genes} \]

2) Identified a transcriptional co-activator, fly ortholog of the yes-associated protein (yap)

   \[ \text{WW domains} \rightarrow \text{interact with PPXY motifs in warts} \]
What is the loss of (ex) or gain of (ex) phenotype of d-yap?

1) Gene overexpression:

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<td>UAS-yap</td>
<td>Act&gt;c</td>
<td>Gal4</td>
<td>off-1</td>
<td>UAS-GFP</td>
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What is the phenotype?

2) Loss of (ex) – "Ends-in" gene targeting (Xie & Golic, 2004)

NOW, CRISPR/TALENS

a) phenotype: Tiny clones; △ prolif; △ cell death a uas-ex uas-eyp,
b) Epistasis: Loss of (ex) yap epistatic to loss of (ex) in wts, hop, sav, x melts...

Ex \( \nearrow \) Hpo \( \rightarrow \) wts \( \rightarrow \) yap \( \rightarrow \) cell prolif (cyce)
| Mer | Hpo | wts | yap | cell death (9 DIAP) |

Molecular epistasis - Example: What is the effect of loss of ex+mer (ex) on expression of expanded?

Experiment:

- UAS-ex \( \rightarrow \) mer^4
- UAS-GFP
- Gal4
- Hpo

Results:
1) \( \nabla \) mer + \( \nabla \) ex (ex)
2) hyperactivation of hop is epistatic to this effect.
2) Increase hpo signaling + follow ex mRNA expression

Result: hyperactivate hpo $\Rightarrow$ increase in ex mRNA expression

hi levels of pathway activity:

ex

ferm

hpo/sau in mohs/wts + yki $\Rightarrow$ yki activated

yki activated (via ex)

lo levels of yki $\Rightarrow$ yki activated (via ex)

Expanded Drosophila cell cycle

What is a limitation of the FLP/FRT system?

Clones are negatively marked.
Goal: Positively-marked mutant clones


1) GAL4 Transactivator
2) Gal80 - corepressor that blocks GAL4-mediated transactivation

MARCM System: hs-Flp UAS-GFP

Any promoter: often Tubulin - GAL4
Wallerian Degeneration:

Axon

Gene activity:

1) Wallerian degeneration appears to be an active process and requires...
2) mutants in hrdS greatly slows Wallerian degeneration
3) No other genes known to be required for Wallerian degeneration

Genetic screen to identify genes required for Wallerian degeneration:

1) What is your assay?
2) How screen: MARCH screen

a. Create a set of 2000 lines that contain independently mutagenized 3rd chromosomes.

b. Gal4 Driver: Os-Gal4: expressed in 20 olfactory neurons per antennae: most if them arise from different lineages.

c. Ex-Frip: expressed in antennae

d. Approach: 1. Use MARCH to induce and follow mutant clones in antennae

2. Sever axons in R but no L antennae

3. Do axons degenerate (all green axons = mutant)
MARCM Cross

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1) Collect ♀ adults
2) MARCM clones generated throughout the eye-antennal disc (green)
3) Only visualize mutant cells under 2 conditions:
   a. cells have undergone mitotic recomb → homozygous mutant
   b. cells express Or22a-GAL4
4) Ablate axons @ Day 1 → cut antennae axons
   ↓
   Wait 7 days (wt axons → degenerated)

Results: Identified 3 non-complementing alleles
- Qtype: no axon degeneration

1) How i.d. gene?
   a. map to small chrom. region → genetically
   b. sequence (WGS)
      - one gene mutated in all 3 backgrounds
   c. Gene rescue: Or22aGAL4 > UAS.dshRNA
      - complete rescue.
How to create library of randomly mutagenized lines

\[ \text{EMS} \]

\[ \text{Or22a-Ga414, UAS-cd8GFP; FRT2A-FRT82B} \times \text{Or22a-cd8GFP; FRT2A-FRT82B} \]

\[ \frac{\text{Dr} \text{ TM3B}}{\text{TM3B}} \]

Single male crosses:

\[ 2000 \times \left( 10^6 \text{Or22a-Ga414, UAS-cd8GFP; FRT2A-FRT82B} \times 3 \text{Or22a-cd8GFP; FRT2A-FRT82B} \right) \frac{\text{Dr} \text{ TM3B}}{\text{TM3B}} \]

\[ \text{self/sib cross} \]

\[ \text{males + females} \]

\[ \text{to establish stock} \]

\[ \text{Library of 2000 independently mutagenized third chromosomes.} \]
The Basics of Next Generation Sequencing

1) PCR-based sequencing

A) Key features:

1) Y adaptors:

```
      5' red
          
      3' green
```

2) Soluble PCR Primers: 5' red 3' (identical to one strand of Y adaptor)

```
      5' green
          
      3' reverse comp. of bottom strand of Y adaptor
```

A lawn of

3) PCR primers covalently attached to flow cell (chemically modified primers)

- identical to soluble primers w/ one exception

```
      5' 3' 8-oxoguanine (cleavable)
          
      3' 2'-deoxyuridine (cleavable)
```

4) DNA Gymnastics
Sheared Genomic DNA

\[ \downarrow \]
+ Soluble primers

\[ 5' \rightarrow 3' \] is reverse complement of green strand of adaptor

\[ 5' \rightarrow 3' \] is identical to top (red strand) of adaptor

\[ \downarrow \]

PCR Amplification

[Diagram showing PCR amplification process]

\[ \downarrow \]

many more PCR cycles

\[ \downarrow \]
size select (1300-2000bp)

\[ \downarrow \]
denature

\[ \downarrow \]
hybridize to flow cell

\[ \downarrow \] Extend → wash off original template → Bridge amplification
Bridge Amplification + Extension

Repeat Many Times

Cleave Top of Red Primer, which has 5'-oligomer 2 enzyme Wash

Add Sequencing Primer
+ G-Fluor
A-Fluor
T-Fluor
C-Fluor

Red Green
Red Green
Red Green
Red Green

for paired-end sequencing; just repeat bridge amplification and cleave green primer.