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Identification of EMS-induced Mutations in Drosophila by Whole Genome Sequencing

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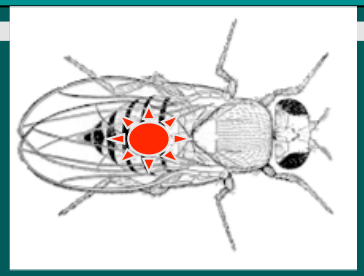
Forward Genetics: Mapping a Muatation(s)



Initial mapping to a chromosome Using Bal stocks

↓

Fine mapping to a region of a chromosome using Df stocks

↓

Select candidate genes

↓

Amplify and sequence genes

↓

Mutation

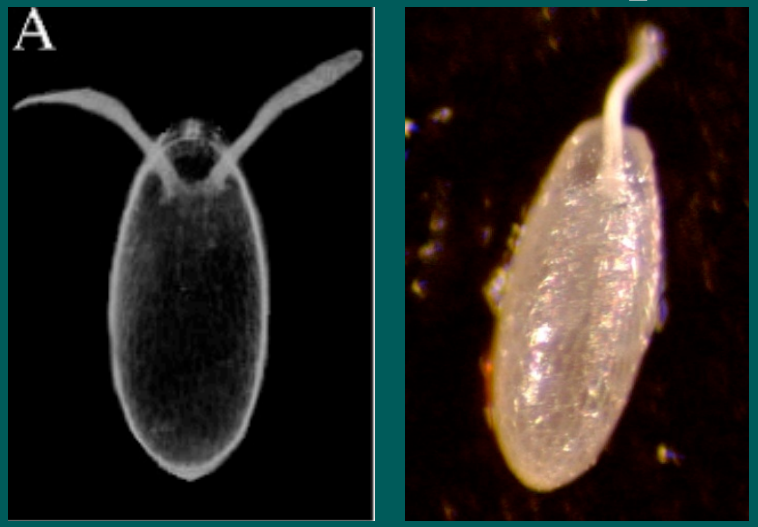
- Labor intensive
- Time Consuming (over 6 months)
- Often difficult
- Sometimes costly

Next Generation Sequencing?

EMS and "Dorsal Appendage Phenotype"

Ethylmethane Sulfonate (EMS)

- Common chemical mutagen
- Randomly induces single base pair mutations or small insertions or deletions
- Usually generates G/C to A/T point mutations



Wild Type **Mutant**

"Mutant Hunt": Using Illumina GA

1. Obtained genomic DNA: F3 homozygous mutants and wild-type males.
2. Performed the following steps:

   a) Made two Illumina genomic fragment libraries (1.5 day)

   b) Hybridized the fragments on to two flowcell – "cluster generation" (2 day)

   c) Sequenced the flowcells (1-2 weeks)
3. Analyzed data to find mutations.

May 2008 using an Illumina GAI

Data Analysis : Primary Analysis

Primary Analysis using Illumina data analysis pipeline.

Images

Sequences

Alignment using ELAND

Uniquely mapping sequences

Data Analysis : Secondary Analysis

1. Aligned each strain individually to reference genome using MAQ (LI *et al., 2008*)

2. Generated consensus sequences for both mutant and wild-type.

3. Compared the consensus sequences of both mutant and wild type strains to generate a list of polymorphisms.

4. Annotated the polymorphisms to find possible mutations causing phenotype.

Run Statistics

	36bp Single Reads (millions)	Base Pairs (millions)	Genome Coverage	% Error Rate
Wild Type (7 lanes)	30	1080	8.7X	0.84 +/- 0.05
Mutant (7 lanes)	29	1044	8.3X	1.14 +/- 0.07

- We were able to cover 71% of the genome at a quality good enough to call mutations!

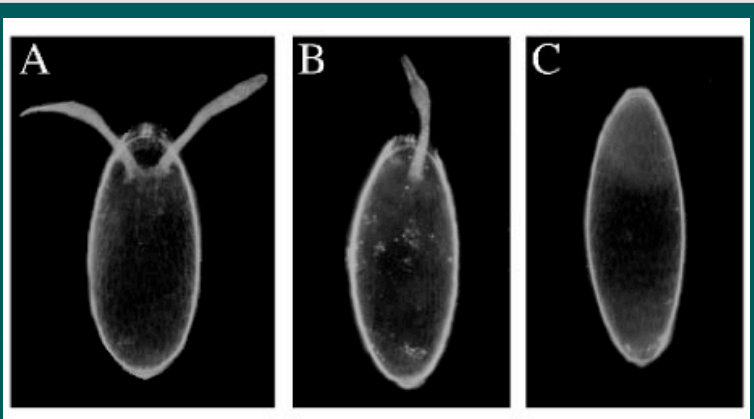
May 2008: Using Illumina GAI

“Needle-in-the-haystack”

165 third chromosome SNPs that are specific to the mutant

Type	Number
Non-coding	143
Synonymous	12
Non-synonymous	8
nonsense	2
TOTAL	165

Nonsense Mutation #1- Encore



HAWKINS *et al*., 1996, 1997; VAN BUSKIRK *et al*., 2000; OHLMEYER *et al*., 2003

-Plays a role in regulation of cyclin E during oogenesis

-Known to have an effect on dorsal appendage formation

-Mutation results in the replacement of a glutamine with a stop codon

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Validation:

Complementation crosses with Df stocks resulted in the same “dorsal appendage phenotype”

Why is this significant? What does this mean to the Drosophila community?

Future of Drosophila genetics?

Step	Traditional Mapping	Whole Genome Sequencing Approach
1. Introduce Mutation	Same	Same
2. Screen for phenotype	Same	Same
3. Narrow down region	Approx. 3-6 months	Not necessary
4. DNA Sequencing	Approx. 1-6 months	Approx. 1-2 weeks
5. Analysis	Approx. 1 months	Approx. 1-2 weeks
Total time spent	6 months to a year!	1 month!

"Well, if I need to find a mutation right now, I would not use traditional mapping techniques, I will find the mutations by sequencing the whole genome because it has been done and it works!"

- Dr. Scott Hawley

Current and Future mutation discovery efforts

- Repeated approach for 3 more Drosophila labs
- Tried approach in C. elegans
  - 2*36bp Paired-end lanes =>20X coverage
  - 2 interesting mutations found!
  - In validation phase
- Genomic capture methods to find mutations in larger organisms.

In Conclusion…

Next generation whole genome sequencing approach can be used to easily map EMS-induced mutations in organisms.

- Cost-effective:

  - Currently (September, 2009), 2*36bp PE lanes> 20X coverage of the Drosophila or C. elegans genomes.

- Time efficient:

  - Opposed to the traditional ways of mapping, mutation(s) can be discovered in about a month!

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Blumensteil *et al*., (2009) Genetics 182:25-32