Model systems

Which of these are genetic model systems (meaning there are mutants that you can use to study aspects of biology)? See if the students know that answer to this. Xenopus laevis (the frog) is not because it is a tetraploid-to get a mutant in a recessive gene, you would have to lose both copies of both genes. There are actually chicken mutants, but almost noone uses them because chickens are just really big, and so it is hard to have enough of them to do genetics. Flies (Drosophila), worms (C. elegans), zebrafish, and mice are all genetic model organisms.

Goal for these classes:

Learn about forward genetics by carrying out a "mock" F3 screen in zebrafish

It is "mock" screen because we are starting with fish that we already know are carrying mutations

Forward Genetics

- Start with a phenotype and then find out what gene(s) mutation is causing the phenotype

- For instance, if you were interested in eye development, you would look for fish with abnormal eye phenotype. You would then identify the gene mutation that caused this phenotype.

Reverse Genetics

- In reverse genetics, you start with a gene and then find out what phenotype results when this gene is mutated.

- For instance, you might start with a gene expressed strongly in the eye, make a knockout mouse that does not express this gene, and see if your mice have any eye defects.
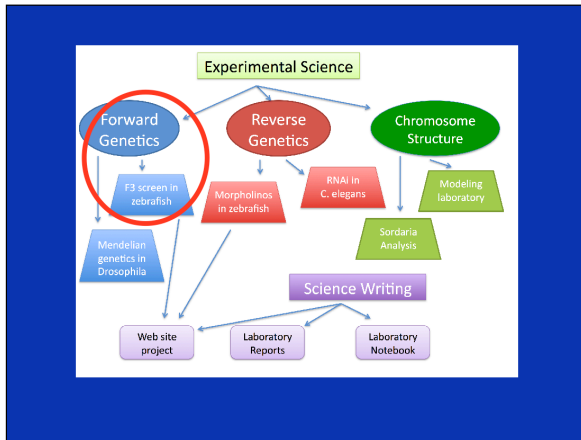
Zebrafish have a unique combination of advantages

1. It's a vertebrate (and so fairly close to humans)
2. Translucent embryos that develop externally
   -can observe development from first cell division
   -follow development in live fish
   -easy to carry out manipulations during very early development
2. Rapid development
   -experiments take less time
3. Relatively inexpensive forward genetic screens

Translucent embryo:

http://zfin.org
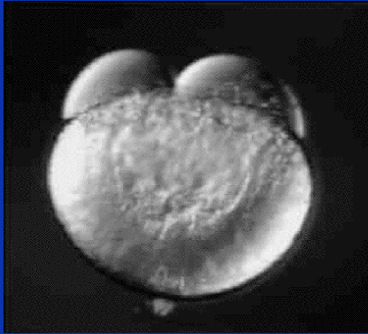
Zebrafish advantages

1. Vertebrate
2. Translucent embryos that develop externally
3. Rapid development

4. Forward genetic screens
   -you can keep enough fish within a single laboratory to do targeted screens-screens for a specific phenotype
   -you can get >100 embryos from each pair of fish (each week!), enough to screen for phenotypes
   -zebrafish are much less expensive than mice

Types of forward genetic screens in zebrafish

1. Haploid screens

2. Early Pressure screens

3. Filial generation 3 (F3) screens

We are going to do part of a "mock" F3 screen. It is "mock" because we know that our tanks contain fish carrying mutations that affect development.

Haploid Screen

2.

ENU

P_O $*/+ \male$ X $+/+ \female$

F_1 $*/+ \female$ UV

X

F_2

haploid embryos
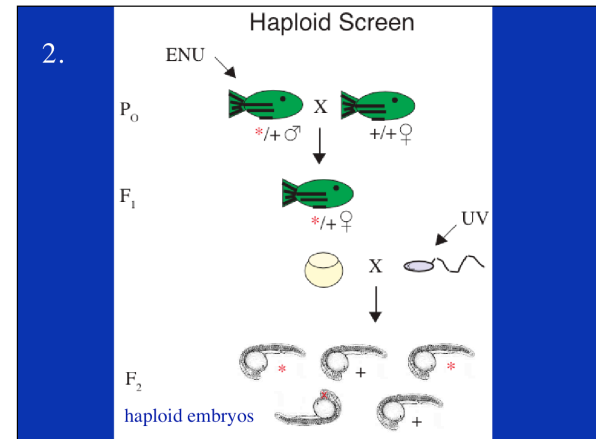
The first step is the same, mutagenized males are crossed to WT females. The female progeny only then is "squeezed" to obtain unfertilized eggs. These eggs are then fertilized in vitro with UV irradiated sperm. The UV destroys the DNA of the sperm so that they are not contributing any genetic information. The resulting progeny are then haploid embryos.

Haploid screen

-No need for F2 generation, so you can screen lots of fish
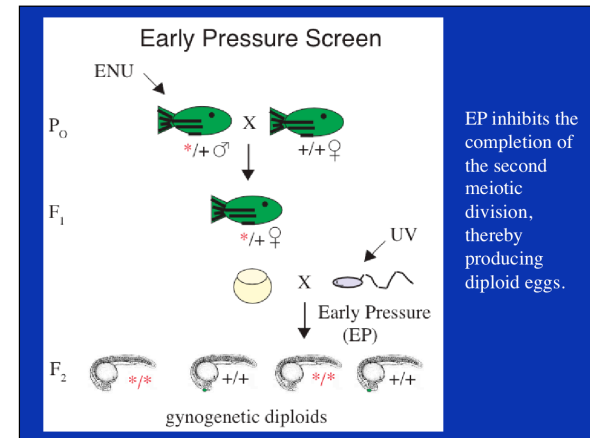
 100 large tanks X 60 fish/tank

 =600 females

-Downsides

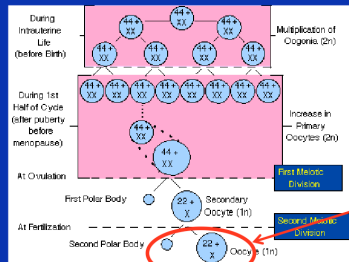 haploid fish live only about 4 days

 have a "haploid syndrome" that affects many structures

 many dead fish, and non-specific defects

 in vitro infertilization is labor intensive

Early Pressure Screen

EP inhibits the completion of the second meiotic division, thereby producing diploid eggs.

EP inhibits the second meiotic division

These two cells are forced back together

http://www.emc.maricopa.edu/faculty/farabee/BIOBK/BioBookmeiosis.html

Remember-in zebrafish there are several germline stem cells (the 44 + XX cells at the top of the pink box) dividing at one time, so a clutch of eggs will come from the oocytes that came from several of these germline stem cells.
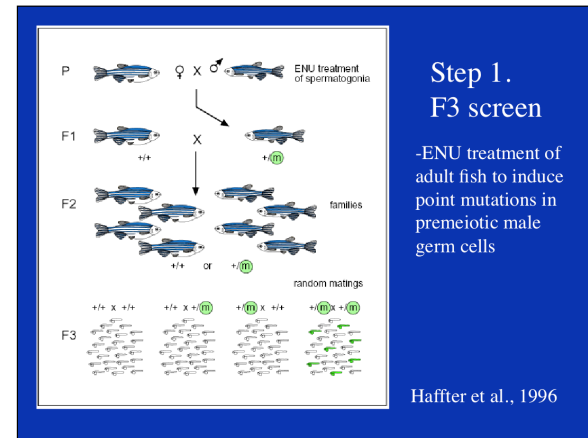
Early pressure (EP) screen

Advantages

-don't have to raise F2, so you can screen lots of fish

-get diploid embryos, so you get "true" phenotypes

-can even screen for adult phenotypes

Disadvantages

-EP is labor intensive

-some non-specific defects

-bias against mutations that are on distal part of chromosome-for distal genes you will get more heterozygotes (up to 96%)

range: 50-4% homozygotes

Most zebrafish mutants were
isolated in F3 screens

Step 1.
F3 screen

-ENU treatment of
adult fish to induce
point mutations in
premeiotic male
germ cells

Haffter et al., 1996

2. Mutagenized males are crossed to WT females

3. These progeny (the F1 generation) are raised to adulthood and then crossed to WT fish in single pair matings (one female X one male)

4. The progeny of each pair (the F2 generation) are raised together as a family. These families are a mixture of fish carrying the mutation (indicated by green m) and fish that are not.

Haffter et al., 1996

The part you will do

The siblings in a family are intercrossed in single pair matings to generate the F3 generation. The F3 generation fish are screened to see if they have a phenotype (like turning green? in this illustration)

Haffter et al., 1996

Ask the students-therefore will all of the clutches you look at today have embryos with a mutant phenotype??

Really big F3 screen

Example:
Haffter et al., 1996 (Nusslein-Volhard laboratory-she
won a Nobel prize for her work in Drosophila)
-screened for morphological defects at 2, 4, and 6 days
-2746 families screened
-18 notochord mutants and 12 mutants that affect
the notochord indirectly
(notochord is probably easiest structure to see)

Each family takes one large tank
If wanted to find 5 notochord mutants, how many tanks
would we need?

Our Plan: Three day protocol

Day 1: Set up male and female F2 fish in
single pair matings

Day 2: Harvest fertilized eggs and sort them
(get rid of debris and bad eggs, count)

Day 3: Screen embryos/larva for
phenotypes

Chi-square analysis and P values

- Chi-square analysis compares the actual values that you get from your analysis of the clutches of zebrafish embryos with the values expected if your hypothesis is correct. Your hypothesis is called the "null hypothesis"

- It is very important to understand the meaning of the P-value that you get at the end of your calculations. P=the probability that the differences between the expected and observed values are due to chance variations.

- P=1 means there is a 100% match between expected and observed values- this would provide very strong support for the null hypothesis, but does not prove the null hypothesis.

- Biologists have come to the consensus that if $P \leq 0.05$ the null hypothesis is rejected. This means that there is an less than or equal to 95% probability that the differences between the expected and observed values are NOT due to chance variations.

Why doesn't a P value of prove the hypothesis? Here is an example-what if you make the prediction that a piece of bread with butter on it will always fall butter side down. You drop the bread two times, and it falls butter side down both times. This give you a P value of 1. Would you bet $1,000 the bread will fall butter side down on the next fall?