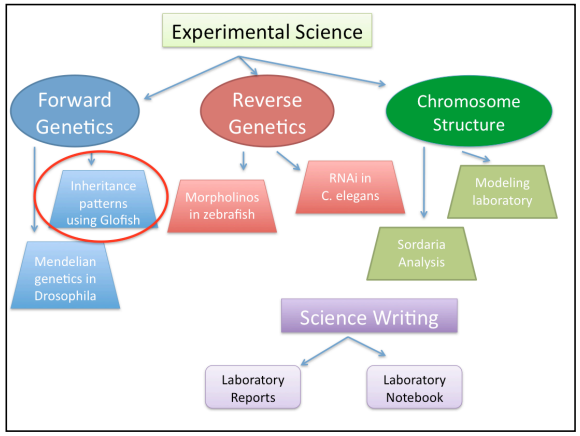


To introduce differences between forward genetics and reverse genetics-suppose you are interested in eye development

Forward genetics: mutagenize an organism that has eyes to induce genetic changes, and then screen progeny for interesting phenotypes (no eyes, big eyes, eyes in the wrong place, eyes that don't work). When you find an interesting phenotype, then you go find the gene that was mutated.

Reverse genetics: you start with a gene that you think is involved in eye development. You then block the expression of this gene in your organism and determine if this causes a change in eye development, morphology, or function.

Chromosome structure laboratories are to enhance learning on how genes and chromosomes are inherited.

Goals for today

1. Learn about transgenes
2. Practice using phenotype to determine genotype (forward genetics skill)
3. Gain experience keeping an accurate and complete laboratory notebook
4. Learn how to use statistical analysis to evaluate a hypothesis

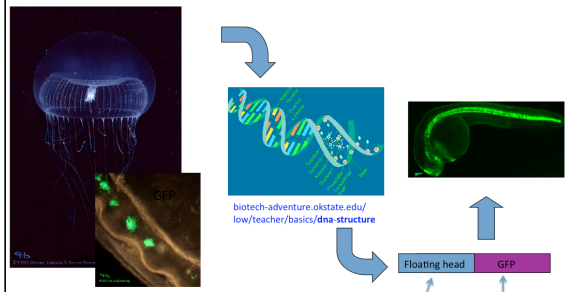
One of the strains we are using today are transgenic zebrafish called GloFish.

They carry genes from jellyfish that encode biofluorescent proteins. They are brightly colored under normal light, and glow in response to specific wavelengths of light.

http:///www.glofish.com

GloFish are the first transgenic animal that is available as a pet.

MAKING A TRANSGENIC ORGANISM
GREEN FLUORESCENT PROTEIN IS ONE OF THE REPORTER PROTEINS USED TO MAKE GLOFISH



http://www.lifesci.ucsb.edu/~biolum/organism/pictures/aequorea.html
Pictures by Steven Haddock and Trevor Rivers

The GFP protein and gene comes from a special strain of jellyfish. The GFP gene can be inserted into the zebrafish genome (you just inject many many copies of the gene-made in the lab-and hope they integrate into the genome). In this case, the gene has the protein coding region for GFP and the promotor for the zebrafish floating head gene, which drives expression in several tissues, including the notochord-the long green stripe of cells that runs along the whole fish.

What (recent) superhero is a GFP transgenic human??

http://www.megomuseum.com/megolibrary/megoart/images/Aurora_hulk.jpg

Hollywood science: In the most recent Hulk movies, the hulk's father injected himself with jellyfish extract, trying to gain the regenerative abilities of jellyfish. The GFP gene must have been in the extract somewhere, and it inserted itself into his germ cells. His son then because a GFP transgenic.

In the old Hulk TV show, David Banner was turned into the hulk after he exposed himself to gamma irradiation, the same method that was used to make the first zebrafish mutants.

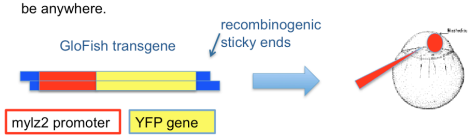
Transgenes

Typically, transgenes are artificial genes made in the laboratory, and are designed to have a specific function.

In zebrafish, transgenes are typically used to mark specific tissues (like the notochord in the previous example)

Transgenes are made in bacteria via bacterial plasmids (circular extrachromosomal bits of DNA), and then linearized and injected into one cell stage zebrafish embryos.

The ends of the injected DNA molecules are recombinogenic, and insert into a chromosome. Important-this is not a targeted event, so the transgene can be anywhere.



We are going to do injections later in the semester

Statistical analysis

- Null hypothesis
- P value
- Chi square test

Null hypothesis

The null hypothesis is the hypothesis that you are testing with your statistical analysis.

In your case it will be a hypothesis about the pattern inheritance of one or more genes.

For example,
The allele that causes zebrafish to have one eye is recessive
The allele that causes zebrafish to have two eyes in dominant

P values

Whatever method you use for your statistical analysis, at the end you will get a Probability- (P-) value.

A P-value is the probability that any differences between expected and observed values are due to chance variation.

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.

P=0.5 means that there is a 50% chance that the differences between the expected and observed values are due to chance.

In biology, we only reject the null hypothesis if $P \leq 0.05$.

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?

Chi-square analysis

- Chi-square analysis is used to test whether your observed values are or are not significantly different from the expected values.
- Therefore the chi-square test can ONLY be used when there are predictable results
- Perfect for Mendelian genetics where you have expected ratios depending upon the type of allele (recessive, dominant, etc.)

For instance
(this is a made up example)

Null hypothesis: recessive mutation and a 3:1 ratio

(1)	(2)	(3)	(4)	(5)	(6)
Phenotype	Observed (o)	Expected (e)	d=o-e	d^2	d^2/e
Two eyes	66	65	1.0	1	.015
One eye	20	21	1.0	1	.048
Curly tail (normal eyes)	1	0*			-
Total					.063

(7) X^2 = the sum of all of the numbers in column 6 = 0.063
(8) Degrees of freedom (df) = n-1 (n is the number of expected phenotypes) = 1
(9) P-value (from table) : $0.7 < P < 0.8$
(10) Conclusion about your hypothesis: Hypothesis is supported

*Note-phenotypes not predicted by the hypothesis should not go into chi-square table