Reverse Genetics in the Mouse
Reading: Ch 6, p 195, 200; Ch 11, p 415; Ch 20, p 721-723

Announcements:
** Problem B at the end of last lectures notes should have read 50 oyster instead of 20. Apologies for the typo – I hope that you were able to logic it out based on the answer given.
** The midterm on Thursday November 6th (7-9 pm) will be held in 2 rooms: 1 Pimentel and 2050 VLSB. You will be assigned to a room depending upon your section. Your assignment will be posted by Wednesday on the website.
** Midterm 2 will cover lectures by Professor Dernburg (9/29 – 10/24) and Amacher (10/27 – 11/3).
** My office hours this week will be held on Wednesday, from 10:15 – 11:45 am.

First, we reviewed zebrafish “half-tetrads” (gynogenetic diploids), which are diploid zebrafish in which all the chromosomes are derived from mom.
(A = centromeric marker segregating in cross; B = gene a certain distance from the centromere, with a recessive allele b)

<table>
<thead>
<tr>
<th>Replicated chromosomes (beginning of meiosis)</th>
<th>No xover in Meiosis I</th>
<th>Meiosis I products</th>
<th>Meiosis II BLOCKED</th>
<th>“Half-tetrads” (non-recomb)</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1.png" alt="Diagram" /></td>
<td><img src="image2.png" alt="Diagram" /></td>
<td><img src="image3.png" alt="Diagram" /></td>
<td><img src="image4.png" alt="Diagram" /></td>
<td><img src="image5.png" alt="Diagram" /></td>
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A question came up after the last lecture about “half-tetrads” – why are they called this and why do we halve of the number of recombinant half-tetrads to get the gene-centromere distance? We answered this question last time by saying “Because only one of the two chromatids in each half-tetrad resulting from a single crossover event is a recombinant chromatid, we must figure that the frequency of recombinant chromatids is half the frequency of recombinant half-tetrads”.

Another way to think of this is to consider what would’ve happened if our example above was a normal yeast meiosis. For example, if the Meiosis I products after a SCO underwent a normal Meiosis II, you would end up with a “tetratype” tetrad, which contains 2 haploid spores with recombinant chromosomes and two without. Remember that to account for this, we halved the number of SCO tetrads in our equations to estimate distance between 2 markers.
Sex determination in mammals
Before investigators actually identified the molecular nature of SRY (for ‘Sex-reversed, Y’ or ‘Sex-determining region, Y’), it was defined genetically and mapped to the Y chromosome, mainly by a series of aberrant X and Y chromosomes that had been generated by “illegitimate” recombination between the X and Y chromosomes during meiosis. Normally, even though X and Y chromosomes pair during meiosis, they rarely exchange information since most of the DNA is non-homologous between the 2 chromosomes. However, occasionally a recombination event does occur, and if it involves the region on the Y that determines “maleness” (SRY), it can generate a sperm in which the Y chromosome is deleted for the SRY gene or the X chromosome with an SRY gene addition.

If a normal egg is fertilized by a sperm carrying an X chromosome with SRY added:
   Result: an XX individual that develops as a male (sterile because other Y-linked genes are required for male spermatogenesis)

If a normal egg is fertilized by sperm carrying a SRY-deleted Y chromosome:
   Result: XY individual that develops as a female. Although these females do have a uterus, they do not fully develop ovaries, thus at puberty, they do not develop females secondary sex characteristics, nor do they menstruate.

SRY encodes a testis-specific transcription factor. How did investigators go from the genetically identified gene interval to the actual gene? First they looked at potential candidates in the interval; one looked particularly good because it encoded a product that was expressed exclusively in the gonadal region before the “unisexual” gonads begin to differentiate either as male or female. Then, by delivering the entire SRY gene to fertilized eggs, where 1 to several copies integrate randomly into the genome, investigators showed that XX karotyped individuals developed as males. In combination with the interval defined by recombination, this was strong evidence to support that SRY was indeed a testis-specific transcription factor.

(See Figure 11.21A for a diagram showing how the transgene is introduced into mice.)
What if one had a candidate gene for a function, but no mutations in that gene to show that loss of gene function causes the expected phenotype? Overexpression or ectopic expression, like the SRY example above, show what a gene can do, but not necessarily what it does do.

Forward genetics: using mutational analyses to uncover gene functions through phenotype (i.e., letting the organism reveal what genes are required)
Reverse genetics: discovering phenotype by directly mutating a specific gene of interest

In the late 1980s, mouse embryologists and geneticists discovered that genes could be modified using homologous recombination to generate all sorts of new alleles by manipulating the genome of mouse embryonic stem (ES) cells. A rare homologous recombination event is selected for (because one disrupts of the gene of interest with a selectable marker by neomycin resistance (neo<sup>R</sup>). The selected ES cells are injected into mouse blastocysts and transferred into foster mothers to generate chimeric mice consisting of wildtype and genetically modified cells. If the germline of the chimera contains some of the modified cells, then the knockout allele can be propagated genetically. See Figure 20.05 below (and in your text).
This part is only for those of you who are curious... (we did not cover it in lecture and I don’t expect you to know, but since some of you asked...)

Injection of the donor molecule that is used for homologous recombination can integrate randomly into the genome. By including a “negative” selectable marker outside the region of homology, one can screen for the neoR colonies that do not contain the second selectable marker and thereby enrich for ES cells that have only the homologous gene targeting event you want and do not carry random integrations of the donor molecule. Figure is from the “Mouse Portrait” of your text.