

**Bioc471a/571a Homework 8 - Due at the start of class October 30**

1. (2 pts) What is the key advantage of the Brand and Perrimon Gal4 enhancer trap strategy over the original method described by Gehring's group? How is the recently described GeneSwitch version of the enhancer trap strategy even more versatile?

**The key advantage of the Brand and Perrimon Gal4 enhancer trap strategy is the ability to regulate the expression of any gene in a cell-specific manner by crossing a Gal4 trapped strain (cell-specific expression) with a strain containing the Gal4 regulatory region upstream of the desired gene.**

**The GeneSwitch strategy is even more versatile because cell-specific expression can be regulated temporally by the addition of RU486 to the food source.**

2. (3 pts.) What is the difference between methods used to improve crop plants incrementally through classical genetics, and the use of transgenesis to create super crops, i.e., can they lead to the same GMO if given enough time? What data supports the view that fields planted with Bt corn could lead to the demise of the Monarch Butterfly, what data refutes this contention? (use links in lecture notes).

**The primary difference between classical genetics and transgenesis with regard to plant breeding is the ability to add non-plant genes to the plant genome. For example, genes encoding an enzymatic pathway to produce a vitamin or nutrient not found in corn or rice. Classical genetics and transgenesis do accomplish the same outcome if the genes involved are from the same species, for example, more fruit/bushel or drought tolerance.**

**The data indicating that Monarch butterflies are sensitive to Bt toxin come from feeding the toxin directly to the insects in the laboratory, usually at much higher doses than would ever be encountered in the wild. The data refuting this result is based on field studies in which natural populations of insects around corn fields were monitored over more than one growing season and shown not to be impacted by pollen dispersal.**

3. (3 pts.) What would explain the lack of gene expression of a randomly integrated transgene in a founder mouse that tested positive by DNA analysis through several generations? What is the most likely explanation of no overt phenotype in a homozygous mouse KO, i.e., the KO mouse is normal despite loss of expression of the targeted gene? How is it possible to maintain a KO strain of mice in which the gene disruption is lethal?

**Random integration events can lead to gene silencing if the local chromatin or nuclear scaffolding interfere with the transcriptional machinery.**

**The most likely explanation for lack of an overt phenotype in a KO mouse is that other gene functions compensate for loss of the targeted gene, i.e., a redundant gene with overlapping activities.**

**Strains harboring homozygous lethal mutations are carried as heterozygotes which are then crossed to do an experiment on the nature of the lethality.**

4. (2 pts.) What is the advantage of the Cre-lox system for generating KO mice over the standard gene replacement strategy? Why doesn't the Cre recombinase protein cause DNA damage in mouse tissues in which it is constitutively expressed, e.g., in the brain of CaMKII-Cre founder mice as described in the lab practicum?

**The advantage of the Cre-lox system is that gene deletions are cell-specific based on cell-specific expression of the Cre recombinase (analogous to the Brand and Perrimon Gal4 enhancer trap strategy).**

**The Cre recombinase does not alter DNA randomly, it only recognizes a specific DNA sequence (lox) that is not found in the mouse genome.**