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**MGCB 31400
AND
BIOS 21236
Genetic Analysis of Model Organisms
Fall 2017
Problem Set #2**

Due Monday, October 23rd in class

We will not accept late problem sets.

Please answer each question in the space provided using LEGIBLE writing. If you need additional space, please use the back of the same sheet ONLY. If you would prefer to type up your answers, the assignment will be available on the Canvas site the day it is distributed. Please print SINGLE-SIDED however.

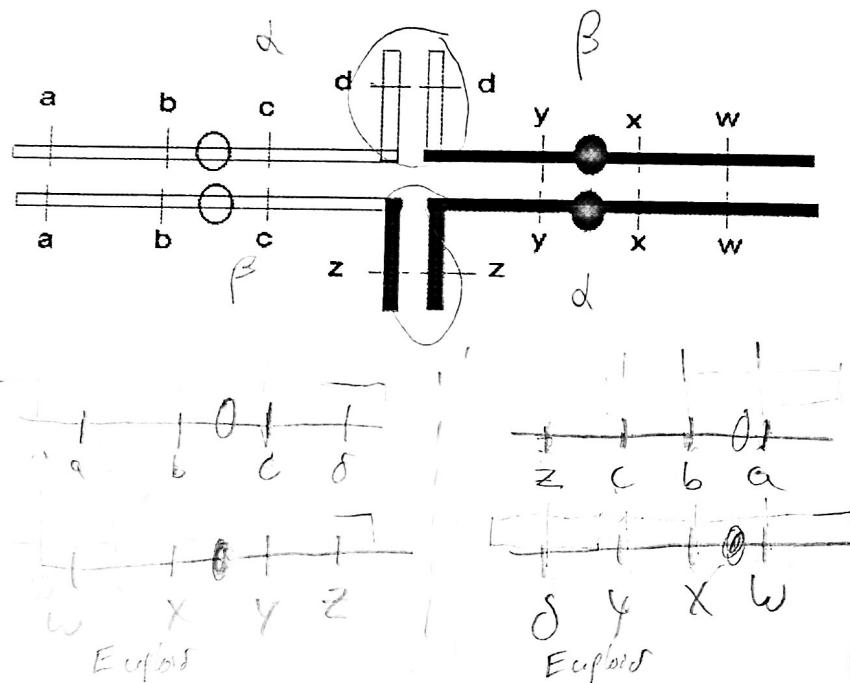
Please write your full name on the top of each of the 11 pages to assist grading.

If you have questions or concerns regarding this problem set please email Philipp at philross@uchicago.edu

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Question 1

If a male fly is heterozygous for a translocation, what fraction of its progeny will survive if it is mated to a female heterozygous for the same translocation? What fraction of surviving progeny will be heterozygous for the translocation? Assume that all aneuploid progeny die as embryos. The figure below shows the pairing of the translocation bearing chromosomes with their normal homologs at meiosis I (sisters are not shown). Using this figure as a guide, diagram the reasoning for your answer to the above question. Assume no recombination and no "adjacent 2" segregation.



1. $\frac{1}{4}$ Alternate Segregation Survive $\therefore \frac{1}{8}$ of gametes are viable
 $\frac{3}{4}$ Adjacent 1 Segregation Die $\therefore 50\%$

2. $\frac{1}{8}$ Surviving Progeny are heterozygous
60%

Question 2

With a source of transposase and absence of repressor, P elements can move around the genome.

- a) The excision of many P elements is precise. The wild-type sequence is restored at the former insertion site. How can that happen, given that P elements create an 8 base pair duplication of host sequences at the site of insertion?

• It does this by both homologous DNA repair and gap junction repair.

For the following three parts of the question, assume that transposition happens during the G1 phase of the cell cycle, i.e., prior to DNA replication.

- b) Is there any genetic condition under which the excision of a P element is almost guaranteed not to be precise? What is that condition, and why does this happen?

• In a cell without homologous DNA for the excision site. This is because there is no template for gap junction repair so it ends up being end joining with the 16 additional bases.

- c) If a P element is heterozygous at a given genetic location, will transposition of that P element usually result in a net increase in the number of P elements in the genome? Why or why not?

No, it should not have a net increase in P elements since it is not a retro-transposon. [Are we going to talk about the

- d) If a P element is homozygous at a given genetic location will transposition of that P element usually result in a net increase in the number of P elements in the genome? Why or why not?

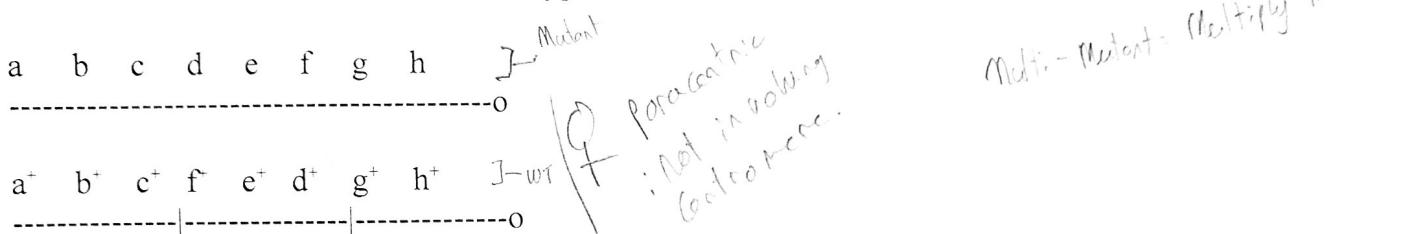
No, the transposon itself is the amalgamation of both 3' & 5' ends.

- e) Does your answer to (c) differ if transposition happens during G2 phase? If so, how?

Possibly, the addition of the extra duplicate chromosomes. If a transposon on the parent strand does excise itself it could end up on the sister chromatid - increasing its genome size. The converse is true for the parent strand.

Question 3

You have a Drosophila female that is heterozygous for an inversion of the X chromosome. (In Drosophila, the centromere of the X is located at one end of the chromosome, thus all X inversions are paracentric.) The chromosome is multiply mutant for a series of marker mutations; the inversion chromosome has the wild-type alleles of all these genes, see diagram below.



This female is mated to a male multiply mutant for the genes a-h. What is the ratio of the number of progeny carrying an X chromosome with a double recombination event within the inversion to the number of patroclinous male progeny?

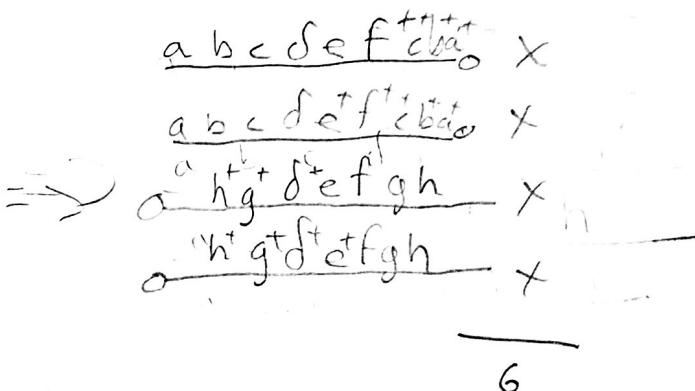
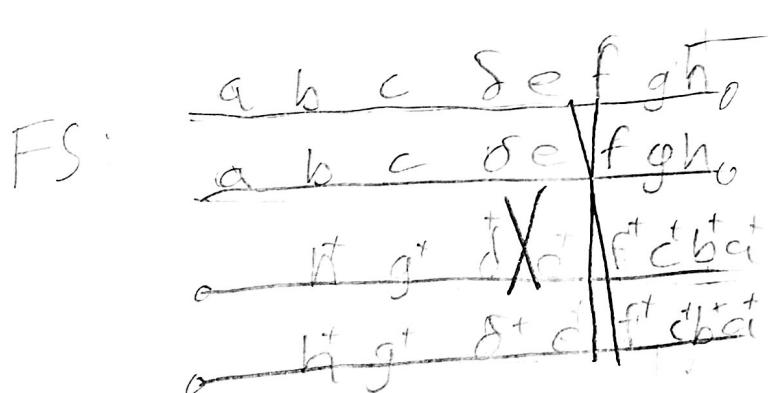
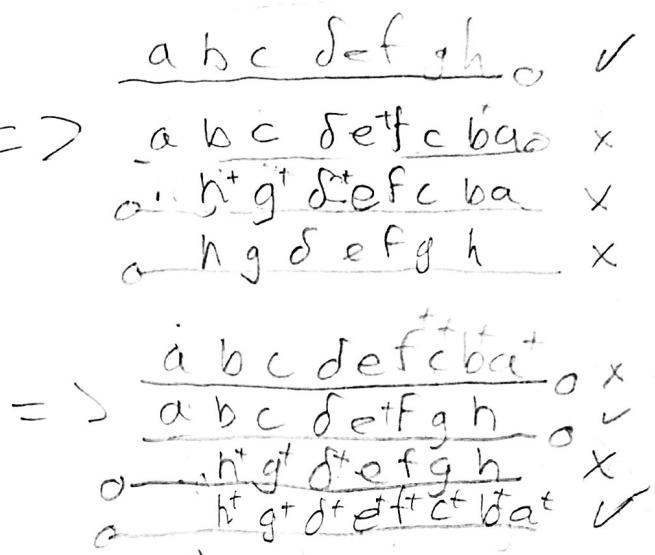
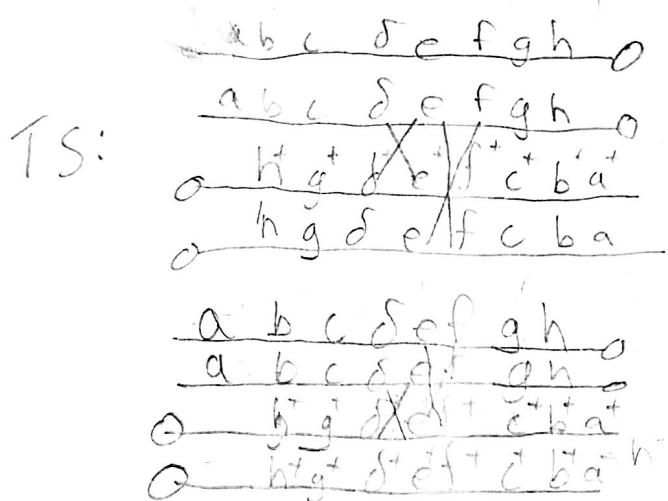
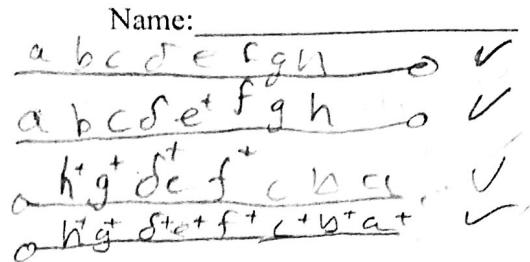
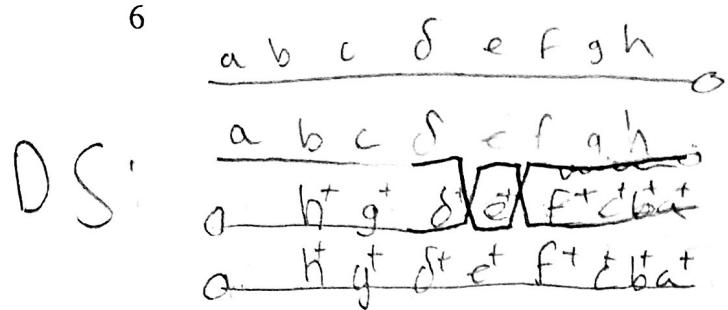
Assume that all double crossover events occur such that one crossover occurs between "d" and "e" and the other occurs between "e" and "f" and thus the viable recombinant chromosomes are visible as either a b c d e⁺ f g h or a⁺ b⁺ c⁺ d⁺ e f g⁺ h⁺ progeny.

Make sure you write the reasoning behind your calculations.

Hints for the logic in addressing this question:

- 1) What is the ratio of the different types of double crossover events – two strand, three strand and four strand?
- 2) What is the structure of the four possible gametes for each of the three types of double crossover events? For each type of double crossover event, what fraction of the double recombinant gametes will be segregated to the oocyte? Remember that normally only one of four meiotic products will be incorporated into the oocyte. However, recombinant gametes that are acentric or dicentric will always remain in the polar bodies; thus, the presence of these classes of gametes influences the chances of a double recombinant gamete being segregated to the oocyte.
- 3) A patroclinous male obtains his X chromosome from his father (and thus displays X-chromosome linked phenotypes characteristic of his father) because his mother did not segregate an X chromosome to the oocyte. However, because such a male did not inherit a Y chromosome, he is sterile.
- 4) Which type of double crossover event in the female could lead to no X chromosome being given to the oocyte (and the subsequent production of patroclinous males)? What fraction of the nullo-X oocytes survives after fertilization by the different classes of sperm?

6



$$\sum \text{Possible Outcomes} = 16$$

x records

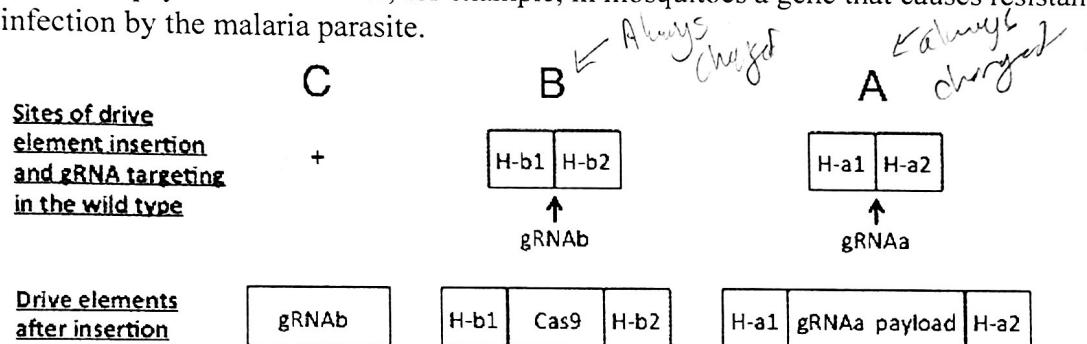
$$\frac{3}{8} = \frac{6}{16} \text{ Viable Chromosomes}$$

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Question 4

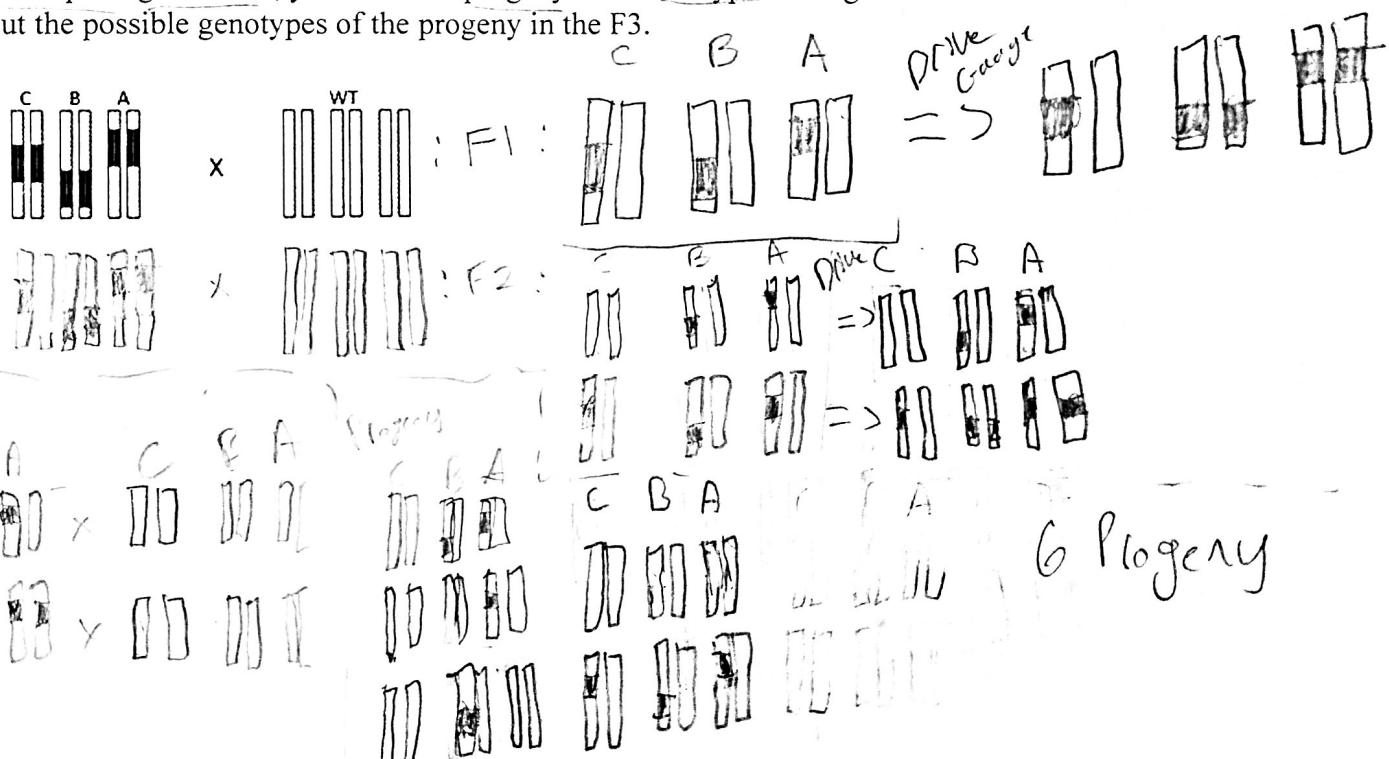
Cas9 mediated gene drives represent a powerful system to modify a species DNA. The danger inherent in this technology is that the drive system can spread throughout the entire species if unchecked. There have been attempts to develop self-contained gene drive systems. One such way to limit the spread of a gene drive is through a system named by its inventor, Kevin Esvelt, a "daisy-chain" gene drive.

A daisy drive system consists of a linear series of genetic elements arranged such that each element drives the next element in the chain. One such daisy drive system is diagrammed below. If the elements are called "A", "B", and "C", then C drives B and B drives A. Assume that A carries a "payload" of interest, for example, in mosquitoes a gene that causes resistance to infection by the malaria parasite.



Assume that Cas9 and the gRNAs are expressed in both soma and germline, each drive element is 100% efficient, the drive elements are not linked, and that homozygosity for a drive element does not cause a deleterious phenotype. Remember that each drive element acts independently.

- a) You have a diploid organism homozygous for each element (represented by black boxes in the diagram below), and you mate that organism with a wild type lacking all drive elements. In each subsequent generation, you mate the progeny with wild type lacking all drive elements. Draw out the possible genotypes of the progeny in the F3.





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payload delivered

- b) If the F3 animals mate with wild type, will all F4 animals have at least one copy of the payload (drive element A)? Why or why not?

No, because there is a ~~progeny~~ with WT genes.

- c) What fraction of the F3 animals is capable of driving the payload to homozygosity in all F4 progeny (assuming that all F3s mate with wild type)?

$$\frac{1}{5}$$

- d) From the above data, what is the general reason why a daisy chain drive does not cause the spread of the payload throughout the entire population?

- It is due to the fact that no matter how many times the drive is active the 'C' element will never become homozygous and the fraction of proper drive function progeny will 1/5 or so.

Question 5

You conducted an experiment in which you first transformed flies with a P element containing a reporter gene (w^+) and a basal promoter next to the coding region for the β -galactosidase gene, lacZ. For each line that contained a P element, you stained embryos and larval imaginal discs for lacZ expression. (Each imaginal disc comprises a small group of cells that are set aside during embryogenesis and that proliferate during larval development to give rise to the ectodermal cells of the adult.)



In one line, you observe that lacZ is expressed in the dorsal region of the wing imaginal disc. You determine that the P element is integrated near a gene expressed in the same pattern as lacZ. You name this gene *apterous*. However, the P element does not disrupt the activity of this gene.

Some view of fly gone

- a) In the strain with the P element, why is lacZ expressed in the same pattern as *apterous*?

Due to it being directly adjacent to a gene with a basal promoter allows it to be expressed alongside the gene of interest whenever the gene of interest is expressed.

- b) You wish to determine whether the *apterous* gene affects patterning of the wing imaginal disc. However, there are no known mutations in *apterous*. How could you use the P element insertion in your line to isolate mutations in *apterous*? Detail the genetic crosses you would use in this experiment and describe how you would genetically identify a chromosome potentially carrying a newly induced mutation in *apterous*. What are two of the molecular techniques to verify that the chromosome has an *apterous* mutation?

*One could EMS mutagenize a group of females, screen for the lacZ reporter in the group, remove and isolate all larvae that lack the *apterous* effect, and then let those flies mate with WT and then cross the progeny with themselves. Some of those progeny will be homozygous for the *apterous* mutant [e.g. lack the *apterous* patterning].*

c) Alternatively, you could use CRISPR to make a null mutation in the *apterous* gene. Detail one of the protocols that you could use, paying attention to the guide RNAs and the homology constructs.

- One could construct a guide-RNA with the intent of creating a shifted reading frame, along with a barcoded section. Insert this complex into an embryo at screen for the lack of the wing imaginal discs wild type phenotype.

d) *apterous* encodes a transcription factor and your loss-of function data have shown that *apterous* is necessary for the production of the dorsal cell fates in the wing imaginal disc. (In an *apterous* mutant, cells in that region of the wing disc adopt fates characteristic of the ventral region of the disc.) You wish to test whether *apterous* can be sufficient to specify dorsal fates in the wing imaginal disc. Conceptually, what is the experiment that you would perform? How would you perform this experiment using P-element technology and the UAS-GAL4 system?

- Conceptually this is a yeast - two-hybrid screen that should be used.
- I would start by transferring a "fly" - into a mutant with a P-element containing a gal-4 gene connected to the *apterous* gene with constitutive expression. I would then create other lines with a 'UAS' element and a GFP gene bonded to a multitude of genes responsible for specifying dorsal fates [and a cDNA library created from flies to be used in *in vivo* experiments]. Then cross the gal4-*apterous* mutants to the various 'UAS' transformants. Look for GFP expression in the progeny that follow and where it organizes [i.e. the dorsal cell fates].

Question 6

The screens that Nüsslein-Volhard and Wieschaus carried out identified mutations in all genes that are required zygotically for patterning the Drosophila embryo. However, these screens did not identify all genes involved in embryonic patterning. In particular, the screen was not designed to identify two classes of genes.

- a) The first class comprises genes that display a strict maternal effect phenotype, i.e., the genotype of the mother determines the phenotype of the embryo. These genes are transcribed in the mother and given to the egg as mRNA or protein. Many such genes encode proteins necessary for setting up the axes (anterior – posterior or dorsal – ventral) of the developing embryo and are not used at any other time during development.

Nüsslein-Volhard performed a second round of genetic screens to identify strict maternal-effect mutations. Similar to the screens for zygotic genes, each of the maternal-effect screens focused on one chromosome. Assume that you have a homozygous viable chromosome marked with multiple visible mutations on the chromosome (*vis*) and a balancer for that chromosome (*Bal*) that has a mutation in one of the *vis* genes, i.e., *Bal/vis* can be distinguished from *Bal/+*.

Describe the experimental protocol you would use to isolate strict maternal effect mutations. Be specific about what flies would be mutagenized in the parental generation, and for each subsequent generation be specific about the genotype of the flies you would select for further mating or phenotypic assay.

- I would do this by treating the larvae with EMS to cause mutations in the 'vis' chromosome and would start colonies with a mutagenized larva and the *vis/Bal*. These lines would grow up and mate ~ $\frac{1}{2}$ the larvae is then screened for interesting phenotypes in the larvae. If interesting phenotypes are seen, then breed the progeny that are heterozygous for the mutation [*vis⁰/Bal*] with the same genotype to create a stock of these mutants with maternal effect phenotypes. Sequence them and compare to the *vis/Bal* genotype.

b) The second class comprises genes for which there is both a maternal and a zygotic contribution to gene activity during embryogenesis. The products of these genes are supplied maternally to the embryo, and the genes are also transcribed during embryogenesis. Many of these genes encode proteins that act at many times during development; thus, a fly homozygous for a mutation in such a gene generally dies during larval development. Why is it very hard to identify mutations in this second class of genes by scoring their embryonic patterning defects?

- Because one could not properly identify what the patterns affect is on the phenotype [Death] of the progeny. Dead larvae typically have no patterning indication, no big sick. After which is is very improbable to isolate the mutation. Plus many of these genes encode proteins short of the larvae.

c) Describe a very general methodology (i.e., do not refer to any specific genetic constructs) that could be used to determine the embryonic phenotype caused by a mutation of a gene in this second class.

• One could create a fly larvae that is a mosaic for one of the genes and record the various phenotypes seen.