1) You have created P element transformants of a construct that contains the mini-white gene, which confers an orange eye color in a homozygous white mutant background. For each line you performed the following crosses:

- Select an initial orange-eyed female transformant and cross this male to w; CyO/Sp; TM3/Dp males.
- Select one orange eyed male progeny that carries CyO and TM3; cross this male to females homozygous for white.
- Score the resulting progeny for the presence or absence of the mini-white gene and the two balancer chromosomes. Below are the results you get for each line. What chromosome is each P element in?

a) Stock 4: 126 flies total: 120 are P[w+]: 64 are CyO; 56 are TM3; all are females. 132 are w-: 68 are CyO; 64 are TM3; all are males.

b) Stock 3 - 110 flies total: 114 are P[w+]: 56 carry TM3; none carry CyO 106 are w-: 32 carry TM3; all carry CyO.

c) Stock 2: 108 flies total: 150 are P[w+]: None carry TM3; 80 carry CyO 66 are w-: all carry TM3; 36 carry CyO.

d) Stock 6: 216 total flies 150 are P[w+]: 76 are TM3; 24 are CyO; 100 are female. 66 are w-: all are CyO; 36 are TM3; all are male.

e) Stock 7: 100 flies total 74 flies are P[w+]: 26 are neither CyO nor TM3; 24 are TM3 and 24 are CyO. 26 are w-: All are CyO and TM3

f) Stock 1 - 230 flies total: 157 are P[w+]: >60 are CyO; none are TM3 73 w-: all are TM3; 40 are CyO.
2) Genomic disorders arise due to structural features common to the human genome, which predispose for specific and recurrent chromosomal rearrangements.

a) Given the presence of the specific repeat sequences indicated below, what types of chromosomal rearrangements would arise from intra-chromosomal and inter-chromosomal non-allelic but homologous recombination.

3) The achaete and scute genes encode two basic helix-loop-helix transcription factors. These genes are expressed in identical expression patterns and reside next to each other in the X chromosome about 25 Kb from each other. Both genes promote neural precursor development. An inversion breakpoint, In(1)sc[8], maps to the intergenic DNA between achaete and scute. Embryos homozygous or hemizygous for this inversion express both achaete and scute in half of their wild-type expression patterns, however in this background achaete and scute are expressed in mutually exclusive patterns.

Provide a molecular explanation of this result.
Also, provide an explanation for why In(1)sc[8] flies exhibit wild-type nervous system development in the face of clear changes to the expression of achaete and scute.
4) The hippo/warts signaling pathway is a relatively newly discovered signaling pathway that regulates cell proliferation and cell death in flies. Mutations in hippo, salvador, or warts lead to increased cell proliferation, decreased cell death, and cause at the molecular level a cell-autonomous upregulation of the levels of the Expanded protein (red in figure). The molecular signal that triggers activation of this pathway remains elusive, as hippo, salvador and warts all function in the signal-receiving cell to mediate signal transduction.

In a genetic screen you identify mutations in two new genes (genes A and B) that yield mutant phenotypes identical to those of hippo, salvador and warts. These mutations complement each other as well as mutations in all known members of the hippo/warts signaling pathway. To determine if either gene functions in the signal-sending cell, you use FLP/FRT-based methods to create mitotic clones homozygous mutant for each gene. You use GFP as your cell-autonomous marker to identify mutant clones unambiguously by their lack of GFP expression (a transgene is used to drive GFP under the control of a ubiquitously expressed promotor), and you assess Expanded protein levels within the mutant clones. Being a careful scientist you also create mitotic clones of a wild-type chromosome as your control. The results of the clonal analysis are shown in the accompanying figure. Please answer the following questions.

A) Which gene functions in a cell autonomous manner and why?

B) Which gene functions in a cell non-autonomous manner and why?
C) Which gene is the better candidate to encode the sought after signaling molecule that activates the hippo pathway.

D) 1. What is the genotype of the cells (dark green) marked by arrow #1.
2. What is the genotype of the cells (light green) marked by arrow #2.

(Assume gene B is in the left arm of chromosome 2; please include all possible relevant information: e.g. FRTs, GFP, centromere…).
**Expanded protein:** red
(darker red = more protein)

**GFP:** green;
**Mutant clone:** no GFP

**Merge of GFP and Expanded**

<table>
<thead>
<tr>
<th>Wild type control</th>
<th>Gene A</th>
<th>Gene B</th>
</tr>
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<tbody>
<tr>
<td><img src="wild_type.png" alt="Wild type control" /></td>
<td><img src="gene_a.png" alt="Gene A" /></td>
<td><img src="gene_b.png" alt="Gene B" /></td>
</tr>
</tbody>
</table>

Gene A

Gene B

1. [Gene A](gene_a.png)
2. [Gene B](gene_b.png)
5) Please read the Willecke et al paper (The Fat cadherin acts through the Hippo Tumor-Suppressor Pathway to regulate Tissue size and answer the below questions.

a) What is the evidence that fat acts in the hippo pathway?

b) Please explain the genetic epistasis tests and their logic that place the action of fat within the hippo pathway?

c) What is the evidence, genetic and otherwise, that indicate that fat exerts its effect on the hippo pathway through expanded but not merlin?
6) Read the paper Arur et al, 2009: Multiple ERK substrates execute single biological processes in Caenorhabditis elegans germ-line development.

A) Why do you think these substrates were not identified in the many genetic modifier screens that were carried out in flies and worms to elucidate the downstream components of the Ras-ERK pathway?

B) Making use of the internet and the information in the paper, please define how many Drosophila proteins contain the ERK-docking site identified in the thyroid hormone receptor.

1. Pick one of these proteins and using commonly available tools to identify the mouse protein that exhibits the highest sequence similarity to it; then define if this motif is conserved between these two fly and mouse proteins.

C) Dis3, an RNA exonuclease, was identified as a substrate of ERK in vitro. In collaboration with Swathi, we determined that ERK and CDK1 phosphorylate Dis3 on the same single amino acid in vitro. This amino acid resides in the most highly conserved region of the protein, the catalytic site of its RNA exonuclease domain. Design two series of experiments that test the effect of phosphorylation on Dis3’s biochemical function in vitro and its genetic function in vivo. (Hint: if you are struggling with this question – read: Arur S, Ohmachi M, Berkseth M, Nayak S, Hansen D, Zarkower D, Schedl T. MPK-1 ERK Controls Membrane Organization in C. elegans Oogenesis via a Sex-Determination Module. Dev Cell 20(5):677-88, 5/2011. PMID: 21571224.)
We are conducting a forward genetic screen to identify genes that regulate post-embryonic neurogenesis. We are mutagenizing an isogenic, lethal-free second chromosome that carries recessive visible mutations in cinnabar, brown, and speck. We are screening for mutations that when made homozygous alter the expression pattern of the transcription factor Hb9 in the thoracic region of the nerve cord; normally, Hb9 is expressed in 18 clusters of neurons in this region. To date, we have identified over thirty mutations (or more accurately mutagenized second chromosomes) that when homozygous yield obvious defects to the expression pattern of Hb9 in the CNS. Of note, larvae heterozygous for each of these mutagenized chromosome and the unmutagenized isogenic, lethal-free chromosome display a wild-type CNS phenotype.

One mutagenized chromosome yields an interesting phenotype: a variable number of Hb9 clusters are absent from most larvae homozygous mutant for this chromosome. A representative set of ten homozygous mutant larvae yield the following phenotype with respect to the presence/absence of Hb9 clusters:

1) 6/18 Hb9-positive clusters are missing
2) 1/18 Hb9-positive clusters are missing
3) 12/18 Hb9-positive clusters are missing
4) 18/18 Hb9-positive clusters are missing
5) 3/18 Hb9-positive clusters are missing
6) 5/18 Hb9-positive clusters are missing
7) 16/18 Hb9-positive clusters are missing
8) 0/18 Hb9-positive clusters are missing
9) 9/18 Hb9-positive clusters are missing
10) 13/18 Hb9-positive clusters are missing

a) What is the penetrance of this phenotype?

b) What is the expressivity of this phenotype?

We performed complementation crosses between this mutant line, which we call K146, and the second chromosomal deficiency kit, which contains ~190 individual fly lines that collectively uncover over 95% of the second chromosome. FYI: the K146 chromosome is carried over CyO; all deficiency lines are also carried over CyO. Our results are as follows:

- Df(2L)ED1272: 0/80 adult flies display straight wings.
- Df(2L) ED1203: 13/32 adult flies display straight wings.
- Df(2L)ED1303: 6/17 adult flies display straight wings.

c) How do you interpret these results?
All larvae of the genotype K146/Df(2L)ED1272 lack all Hb9 clusters and exhibit a nerve cord itself much reduced in size relative to K146 homozygous larvae.

d) From these data, what would you interpret about the nature of the affected allele?
   - In your answer, refer both to Muller’s classification scheme for mutant alleles (Tim went over this, and I always ask questions in prelims on this) and to the likely molecular nature of the allele.

Next, we performed a second round of deficiency mapping of K146 against each of six deficiencies yielding the following results:

Df(2L)EXEL6043: 31/76 adult flies
Df(2L)EXEL8041: 0/42 adult flies have straight wings.
Df(2L)EXEL9043: 0/50 adult flies have straight wings.
Df(2L)ED1226: 0/45 adult flies have straight wings.
Df(2L)BSC301: 0/48 adult flies have straight wings.
Df(2L)BSC341: 11/30 adult flies have straight wings.

e) Using FLYBASE (http://flybase.org/), print-out a map of a genomic region that contains the relevant portions of the nine deficiencies mentioned above; on this map, circle the smallest region most likely to contain the relevant mutation. (Hint: Find a genomic map of the relevant deficiency using the following path from the Flybase homepage: Resources>Stock Collections > Bloomington; once you are at the Bloomington stock center page, search for the relevant deficiency).

In parallel, we sequenced the genome of larvae homozygous mutant for the K146 chromosome (as well as that of the isogenic, lethal-free chromosome from which we generated K146 via EMS mutagenesis). The ‘K146” spreadsheet found within the H84-K146 EXCEL file contains all SNPs that were identified to be unique to the K146 chromosome relative to the unmutagenized target chromosome from which K146 was derived (there are two different spreadsheets in this file; make sure you are looking at K146). Please note, coverage of ≥5X is required to call a SNP.

f) Which SNP is the most likely to identify the mutation that when homozygous leads to the observed phenotype?

g) How would you prove this?
8) Through the identical approach, we mapped another mutation, referred to as H84, via deficiency mapping and sequenced its genome to identify SNPs that were unique to the H84 mutagenized chromosome relative to the unmutagenized chromosome from which it was derived. The relevant deficiency mapping results are below, and the EXCEL spreadsheet called “H84” contains the identified SNPs for this background.

Deficiency Mapping results:

- Df(2R)nap9: 0/50 adult flies have straight wings.
- Df(2R)BSC313: 15/50 adult flies have straight wings.
- Df(2R)BSC261: 21/50 adult flies have straight wings.
- Df(2R)ED1552: 0/50 adult flies have straight wings.
- Df(2R)ED1612: 0/50 adult flies have straight wings.
- Df(2R)BSC326: 0/50 adult flies have straight wings.
- Df(2R)Exel6050: 19/50 adult flies have straight wings.
- Df(2R)Exel6051: 23/50 adult flies have straight wings.

a) In what physical location is the relevant mutation most likely to reside?
b) Is there an obvious SNP that identifies the likely causative allele?
c) If not, please outline the steps you would take to identify the affected gene/causative allele?