Question 1. (14 points)
The plant, *Banana examinus*, is diploid and $2n = 4$. There is one long pair and one short pair of chromosomes. Each diagram represents an anaphase stage of an individual cell during meiosis or mitosis in a plant that is genetically dihybrid ($A/a ; B/b$) for genes on different chromosomes. The lines represent chromosomes or chromatids, and the points of the Vs represent centromeres. In each case, determine whether the diagram represents a cell in meiosis I, meiosis II, or mitosis, OR if a diagram is "abnormal" (not correct) indicate this and very briefly explain why.

A) (3 pts) Meiosis I. Partial credit (1 pt) if described as abnormal but close to Meiosis I.

B) (4 pts) Abnormal (2 pts); too many chromatids (or too many chromosomes, or $2n=8$) (2 pts)

C) (3 pts) Meiosis II. Partial credit (1 pt) if described as abnormal but close to Meiosis II.

D) (4 pts) Abnormal (2 pts); sister chromatids should be identical (2 pts). Must mention sister chromatids or mitosis; -1 if termed “homologous” chromosomes. Lack of identity could be described as evidence of recombination or “problem with Aa segregation.”

Question 2 (18 points).
2. The activity of the enzyme beta-galactosidase produced by cells containing certain mutations was measured (in relative units) when the cells were grown in media supplemented with different carbon sources. (Glycerol can be metabolized by these cells but is not a sugar.)

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Glycerol</th>
<th>Lactose</th>
<th>Lactose + Glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wildtype</td>
<td>0</td>
<td>1000</td>
<td>10</td>
</tr>
<tr>
<td>Phenotype 1</td>
<td>1000</td>
<td>1000</td>
<td>10</td>
</tr>
<tr>
<td>Phenotype 2</td>
<td>0</td>
<td>1000</td>
<td>1000</td>
</tr>
<tr>
<td>Phenotype 3</td>
<td>0</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Phenotype 4</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Match the mutant phenotype with the mutation(s) below. (Different mutations may have the same phenotype.) BRIEFLY explain your reasoning for each answer.

*Note: for requirement to “explain your reasoning,” no credit given for restatement of phenotype.*

a) nonsense mutation in the *lacZ* gene:

(4 pts) Phenotype 4 (2 pts); the *lacZ* transcript encodes a truncated or non-functional protein (2 pts). Partial credit (1 pt) if claimed the *lacZ* gene is non-functional. No
credit for claiming that the transcript is truncated. [Must be clear is at level of protein function.]

b) $I^s$

(4 pts) Phenotype 4 (2 pts); (super) repressor constitutively binds the operator (or cannot bind lactose) (1 pt), inhibiting transcription under all conditions (1 pt). No credit if repressor described as “always on” or as repressing beta-gal. [Must be clear is at level of transcriptional regulation.]

c) $O^c$

(5 pts) Phenotype 1 (2 pts); operator mutation (1 pt) that results in absence of negative control by repressor (1 pt), but still under positive control of CAP-cAMP binding (necessary for high levels) (1 pt).

d) nonsense mutation in the $crp$ gene (encoding CAP protein)

(5 pts) Phenotype 3 (2 pts); absence of (positive) control by CAP-cAMP prevents high levels of transcription (in absence of glucose) (2 pts), but negative control (effected by repressor) still functions (1 pt).

Question 3 (14 points).

Two purebreeding strains of mice are crossed to produce F1 mice that are heterozygous for dominant and recessive alleles of three linked genes ($A$ vs. $a$, $B$ vs. $b$, $E$ vs. $e$). The F1 mice are then test crossed and the resulting progeny are:

<table>
<thead>
<tr>
<th>A B E</th>
<th>12</th>
<th>2*12</th>
</tr>
</thead>
<tbody>
<tr>
<td>$A B e$</td>
<td>330</td>
<td></td>
</tr>
<tr>
<td>$A b E$</td>
<td>102</td>
<td>102</td>
</tr>
<tr>
<td>$A b e$</td>
<td>55</td>
<td>55</td>
</tr>
<tr>
<td>$a B E$</td>
<td>45</td>
<td>45</td>
</tr>
<tr>
<td>$a B e$</td>
<td>98</td>
<td>98</td>
</tr>
<tr>
<td>$a b E$</td>
<td>350</td>
<td></td>
</tr>
<tr>
<td>$a b e$</td>
<td>8</td>
<td>2*8</td>
</tr>
<tr>
<td><strong>1000 total progeny</strong></td>
<td><strong>340</strong></td>
<td></td>
</tr>
</tbody>
</table>

a) On the table above, CIRCLE the parental types.

(2 pts). See above.

b) What is the map order of the three genes? Explain your reasoning.

(4 pts) $A$-$E$-$B$ (2 pts) Explanation (2 pts): The rarest classes represent double crossovers; compare the alleles in these classes with those in parents; the allele that differs is in the middle (-1 for omission of any of the 3 points).

c) Using all of the data, what is the map distance between the two genes that are farthest apart?

Show your work below and/or on the table above.

See work above.
(8 pts) \( R_{ab} = \frac{340}{1000} = 34\% \) or 34\mu. If omitted all DCO classes, -4; if failed to double DCO classes, -2; if math error, -1; if expressed as “340”, -2.

**Question 4 (16 points).**

Many of the most effective antibiotics are compounds made by fungi that act by inhibiting bacterial protein synthesis. A new antibiotic, named edeine, has been isolated. Edeine inhibits protein synthesis but has no effect on either DNA synthesis or RNA synthesis. The effects of edeine can be studied using an *in vitro* translational system. By adding purified mRNA for \( \beta \)-globin, protein synthesis can be followed by measuring the incorporation of radioactive amino acids into completed \( \beta \)-globin protein. (See the figure below.)

![Diagram](image.png)

(a) Analysis of the edeine-inhibited solution found that all the globin mRNA was attached to the small ribosomal subunit. Which step(s) in protein synthesis might be inhibited by the addition of edeine? Explain briefly.

(7 pts) Affects initiation (3 pts; -1 pt if not mentioned); the large subunit fails to attach, or problem with locating or binding to initiating Met (4 pts); -1 for correct answer plus additional erroneous possibilities. Partial credit: affects a termination step (2 pts); prevents tRNAs from becoming charged (2 pts); up to 3 pts compensatory credit for general description of translation process.

(b) Why is there a lag between the addition of edeine and the cessation of protein synthesis?

(4 pts) The ribosomes that have already initiated synthesis continue translating until completion (4 pts); general statement that affects a process already initiated (2-4 pts, depending upon level of detail). For the tRNA theory: pool of already charged tRNAs must be used up (2 pts). For the termination theory: translation continues until all ribosomes stalled just prior to termination (2 pts).

(c) In contrast, the addition of cycloheximide causes the immediate cessation of protein synthesis. What possible step(s) might this drug block? Explain briefly.

(5 pts) Blocks any one of the many steps (1 pt) required during elongation, translocation or indexing (2 pts for each one of these terms mentioned; -1 pt if none mentioned) such as … (2-4 pts for description of specific step, depending upon level of detail). Partial credit: destroys the ribosomes (1 pt); causes the ribosomes to detach from mRNA (1 pt).
**Question 5 (8 points).**

A 30 kb region of human genomic DNA includes the following restriction sites for *EcoRI* (symbolized R) and *BamHI* (symbolized B):

```
  B  R  R  B
  2.4 4.0 1.4 7.2 2.6 3.8 2.2 6.4 (sizes in Kb)
```

Two transcripts are encoded in this region of DNA (*transcript 1* and *transcript 2*). The lines above *transcript 1* and *transcript 2* in this diagram indicate the extent of the pre-mRNA (also called the nascent transcript).

A) The 1.4 kb restriction fragment (circled above) is cloned and then used as a probe. If human genomic DNA is cut with *EcoRI*, what size restriction fragment(s) will hybridize to this probe? Show your work.

\[
1.4\text{kb} + 7.2\text{kb} + 2.6\text{kb} + 3.8\text{kb} = 15\text{kb}
\]

(4 pts) 15-kb fragment. Partial credit: 2 pts if included all products of partial digest that includes the 15-kb fragment; 2 pts if 15-kb the predominant, but others slightly (because of ends).

B) The 2.2 kb restriction fragment (boxed above) is cloned and then used to probe a cDNA library made from human skin cells. This probe fails to hybridize to any cDNA clone. What is a possible explanation as to why this probe does not detect a cDNA?

(4 pts) A cDNA library represents transcripts (2 pts) (“contains only exons” or “contains no introns” reluctantly accepted). The transcript detected by the 2.2kb probe may not be made in skin cells, or the region detected by the 2.2kb probe may be within an intron (2 pts).

**Question 6 (16 points).**

The pedigree below shows the inheritance of a rare X-linked recessive trait. The accompanying Southern blot shows inheritance of an RFLP that is 10 map units from the disease gene.
A) Using the above pedigree and ignoring the RFLP data, what is the probability that individual E will have an affected child? Show your work and define any symbols used.

\[ A = \text{dominant wild-type allele} \]
\[ a = \text{recessive disease allele} \]
\[ Y = \text{Y chromosome} \]

- **C genotype is A/a**
- probability of E being A/a = \(\frac{1}{2}\)
- probability of child inheriting “a” = \(\frac{1}{2}\)
- probability of child being male = \(\frac{1}{2}\)
- probability of child being affected = \(\frac{1}{2} \times \frac{1}{2} \times \frac{1}{2} = \frac{1}{8}\)

(6 pts) \(\frac{1}{8}\). 2 pts each component (probability of E being A/a; probability of child inheriting a; probability of child being male).

B) If C and D were to have another son, what is the probability that their son would have the trait? Show your work.

- (4 pts) C genotype is A/a, so probability of passing on “a” allele to son is \(\frac{1}{2}\) (4 pts)
  - (RFLP data does not affect this probability)
- If fail to realize that C must be a carrier, -3; if fail to realize that child being a son is given, -2; if assume recombination an issue at this step, -2.

C) Considering all of the data, what is the probability the individual E will have an affected child? Show your work.

- **C genotype is A 10kb/ a 2kb**
- E inherited 2kb allele from mother
  - probability of also inheriting “a” is 90%
- probability of child inheriting “a” = \(\frac{1}{2}\)
- probability of child being male = \(\frac{1}{2}\)
- probability of E having an affected child = \(0.9 \times \frac{1}{2} \times \frac{1}{2} = 22.5\%\)

(6 pts) 22.5\% (9/40 accepted): probability of E being A/a x probability of child inheriting a x probability of child being male. If error in recombination component, -4; if error in any other component, -3; if included recombination component twice, -2.

If error from part a was propagated, no deduction.

Question 7 (20 points).
A cloned piece of DNA is sequenced using the dideoxy method. The result of this sequencing is shown below on the left. You have strong evidence that this sequence includes the translational start site for a protein 248 amino acids long. However, you do not know if you are directly sequencing the strand of DNA that is transcribed or its complement.
a) What is the double-stranded sequence of this cloned DNA? (Label the 3’ and 5’ ends.)

5’ TACCTAATTAGGCTTCATC 3’ (from gel)
3’ ATGGATTAATCCGAAGTAG 5’ (template)

(5 pts) 3 pts for sequence, 2 pts for properly labeled ends. If included 1 strand only, -2 pts.

b) Which strand must be transcribed (the strand directly read from the gel or its complement)?

(3 pts) Strand read directly from gel is the transcribed strand (look for the AUG start codon in the complementary coding strand). [This is true whether or not strands were reversed in a.]

5’ G AUG AAG CCU AAU UAG GUA 3’

c) What can you deduce about the amino acid sequence that would be coded for by this DNA after it is transcribed?

(6 pts) 2 alternatives accepted: Met-Lys-Pro-Asn-STOP (-1 if aa incorrect, -2 if unspecified) if ends correctly assigned in part a. Met-Asn-STOP (-2 if aa incorrect or unspecified) if ends incorrectly assigned in part a. For hybrid combinations of answers, -1 point for inconsistency.

d) Is there any evidence that you have also sequenced part of an intron? Explain briefly.

(2 pts) Yes. It is stated that the protein is 248 amino acids long yet there is an in-frame stop codon after 4 amino acids (or 2 amino acids, if consistent with part c).

e) Identify a possible restriction site that exists in this DNA sequence.

(4 pts) 10-mer within this sequence: CCTAATTAGG. Any palindromic subset, from 4-mer to 10-mer, either strand, accepted.

**Question 8 (16 points)**

Which of the following statements about embryonic pattern formation in Drosophila are true and which are false? Briefly justify each answer. (Assume all mutations are null mutations.)

a) An embryo homozygous for a pair-rule mutation exhibits abnormal patterns of segment polarity gene expression.
(4 pts) True (1 pt); pair-rule genes are upstream of (epistatic to) segment polarity genes (3 pts).

b) When a male homozygous for a mutation in the bicoid gene is crossed to a heterozygous female, half of the embryos will exhibit abnormal patterns of gap gene expression.

(4 pts) False (1 pt); bicoid mRNA transcribed from the maternal genome (maternal effect gene) (1 pt); since mother has one good copy (1 pt), all embryos will develop normally.

c) One quarter of the embryos produced by parents heterozygous for a pair-rule gene mutation will exhibit abnormal patterns of gap gene expression.

(4 pts) False (1 pt); pair-rule genes are downstream of gap genes (3 pts).

d) An embryo homozygous for a mutation in the homeotic gene Antennapedia (Antp) shows an abnormal number of segments.

(4 pts) False (1 pt); homeotic genes determine segment identity (1 pt), not segment number (segment number determined by pair-rule genes in a different pathway) (2 pts). Statement that a different pathway not required, but if implied otherwise, -1.

Question 9 (18 points).
You have in your possession a wild-type Drosophila strain and a mutant strain that doesn’t make nanos protein. You also have cloned cDNAs for nanos and bicoid. These plasmids are as follows:

<table>
<thead>
<tr>
<th>Plasmid</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>plasmid 1</td>
<td>full length nanos cDNA</td>
</tr>
<tr>
<td>plasmid 2</td>
<td>bicoid 5’ UT + nanos ORF + nanos 3’ UT</td>
</tr>
<tr>
<td>plasmid 3</td>
<td>nanos 5’ UT + nanos ORF + bicoid 3’ UT</td>
</tr>
<tr>
<td>plasmid 4</td>
<td>bicoid 5’ UT + nanos ORF + bicoid 3’ UT</td>
</tr>
</tbody>
</table>

where UT = untranslated region and ORF = open reading frame (protein coding region)

a) The plasmids are constructed so they are expressed in transgenic animals. You systematically create eight transgenic fruitfly strains by introducing each of the four plasmids into the wild type and nanos mutant strains. Then, you look where the nanos protein is spatially localized in the fly embryo. Complete the following table indicating where nanos protein is found in each transgenic strain, answering either Anterior, Posterior, Both, or Neither. You may abbreviate A, P, B, or N for your answer.

(10 pts) One point for each.

<table>
<thead>
<tr>
<th>Plasmid</th>
<th>Transgenic Wild Type</th>
<th>Transgenic nanos mutant</th>
</tr>
</thead>
<tbody>
<tr>
<td>No plasmid</td>
<td>P</td>
<td>N</td>
</tr>
<tr>
<td>plasmid 1</td>
<td>P</td>
<td>P</td>
</tr>
<tr>
<td>plasmid 2</td>
<td>P</td>
<td>P</td>
</tr>
</tbody>
</table>
b) If wild type embryos are grown in the presence of colchicine, a drug that inhibits microtubule formation, where would you expect nanos protein to be located? Explain briefly.

(8 pts) Not localized (2 pts, mandatory); microtubules play essential role in localization (2 pts, mandatory); it is the mRNA that is localized (2 pts, mandatory; 0 if protein; 1 pt if generic “nanos”). Additional 2 points for further details: normally localized to posterior (1 pt); role of UT sequence (2 pts); role of polarity of microtubules (1 pt); transported (1 pt) along the microtubules (1 pt), etc.

Question 10 (20 points).
A mutant screen resulted in large numbers of Neurospora crassa mutants that are auxotrophic for the amino acid arginine. These mutants were then grown on minimal media plates supplemented with chemicals structurally related to arginine. It was found that the mutants could be grouped into three classes, 1-3.

Supplemented with Arg (L-Arginine), all strains will grow
Supplemented with Orn (DL-Ornithine), only class 1 strains will grow
Supplemented with Cit (L-Citrulline), only class 1 and class 3 strains will grow

a) Propose a biochemical pathway for the synthesis of arginine

precursor → Orn → Cit → Arg

(4 pts) -3 if Orn and Cit reversed; -2 if backwards; -1 if omitted precursor.

b) Show the relationship between the steps in the pathway above and the genes in the three classes of mutants.

class 1
precursor → Orn → Cit → Arg
class 3
class 2
(3 pts) If error in a, partial credit if any correct subset.

c) What compound(s) will accumulate in the cells of class 3 mutants?

Orn

(2 pts) Orn (Propagated error – no deduction if consistent with a/b.)

d) A double mutant contains a mutation from class 1 and a mutation from class 3. What type(s) of plates will this double mutant grow on? (i.e. what supplement(s) should be included in the minimal media plates?)

(2 pts) Plates supplemented with Cit (1 pt) or Arg (1pt); -1 if state Cit AND Arg. (Propagated error – no deduction if consistent with a/b).

e) What compound(s) will accumulate in the cells of the double mutant from (d)?

(2 pts) Precursor (Propagated error – no deduction if consistent with a/b).

f) Mutation “x” and mutation “y” are recessive alleles that fall into class 2. How would you determine whether “x” and “y” represent one or two genes? Be sure to indicate how you would interpret your results.
(7 pts) Do a complementation cross, or cross x with y (2 pts). [Note: no deduction if stated simply to plate both on the same plate, since apparently this is all that it takes for Neurospora.] Plate on minimal medium (1 pt), OR statement that this is an appropriate test, since both are recessive (1 pt). If resulting progeny show the mutant phenotype (fail to grow), x and y are mutations in the same gene (2 pts); if they do not show the mutant phenotype (are able to grow), they are mutations in 2 different genes (2 pts). Partial credit: 1 pt each for statements that “if fail to complement are in the same gene” and “if complement are in different genes” without an explanation of what the experimental result is (mutant or wild-type) in at least one case.

Question 11 (20 points).
The wild-type Rb protein functions to sequester the transcription factor E2F in the cytoplasm. At the appropriate time in the cell cycle (G1), a cyclin-dependent protein kinase/cyclin complex phosphorylates Rb, which then releases E2F so that it can enter the nucleus and act as a transcription factor to activate genes required for the S phase of the cell cycle.

Determine what affect each of the following Rb mutations would have on the progression through the cell cycle. Justify your answers.

a) pRb is constitutively phosphorylated.
   (4 pts) More cell division (1 pt) due to premature entry into S-phase (1 pt; any mention of S-phase given credit); pRb releases E2F or fails to sequester E2F (1 pt) and thus … (any further description of consequences: uncontrolled movement of E2F into nucleus; increased transcription of E2F target genes; increased levels of gene products required for DNA synthesis, etc., 1 pt)

b) The phosphorylation site of pRb is mutated, thus it can never be phosphorylated.
   (4 pts) No cell division (1 pt), stalled in G1 (or cannot enter S-phase or enters G0) (1 pt); pRb cannot release E2F or E2F continually sequestered (1 pt) causing …(any further description of consequences: E2F never enters nucleus, genes required for S-phase cannot be transcribed, etc. 1 pt).

c) The E2F binding site of pRb is mutated so that no binding occurs.
   (4 pts) More cell division (1 pt) due to premature entry into S-phase (1 pt; any mention of S-phase given credit); E2F always active (1 pt) because …. (cannot be sequestered in cytoplasm or other description of consequences, 1 pt). “Like part a” accepted.

d) The cyclin A- CDK binding site of pRb is mutated so that no binding occurs.
   (4 pts) No cell division (1 pt), stalled in G1 (or cannot enter S-phase or enters G0) (1 pt); pRb will not be phosphorylated (1 pt) and thus cannot release E2F (1 pt). “Like part b” accepted if role of phosphorylation mentioned.

e) The normal pRb protein is expressed at lower levels than normal.
   (4 pts) Probably more cell division (2 pts) since more E2F is in active (unsequestered) form (2 pts). If mentioned that effect likely to be small (or less than a and c), 1 pt compensatory credit for any points lost in parts a-d. “No effect” accepted if pointed out that retinoblastoma is recessive at the cellular level.

Question 12 (20 points).
The *Drosophila* compound eye is composed of about 800 units called ommatidia. Each ommatidium contains eight photoreceptor neurons (R1 through R8), which develop in a fixed order. Mutations have been isolated that perturb the differentiation of the R7 cells. Two such mutations are in genes called *Sev* (*Sevenless*) and *Boss* (*Bride Of Sevenless*). R7 progenitor cells in fly eyes that do not express either BOSS or SEV (or both) differentiate into non-neural cone cells (instead of R7 cells). Too many R7 cells develop in fly eyes that are overexpressing either BOSS or SEV (or both).

a) Overexpression of SEV protein in fly eyes that do not express functional *Boss* leads to ommatidia with too many R7 cells. Overexpression of BOSS in fly eyes that do not express functional *Sev* generates ommatidia with no R7 cells. What do these results imply about the epistatic relationship between *Sev* and *Boss*?

(2 pts) *Sev* is epistatic to *Boss*, or *Sev* is downstream of *Boss* (2 pts). -1 if stated *Sev* is epistatic to *Boss* but is upstream.

*Explanation (not required for full credit):*

*Whenever phenotype is displayed by the double mutant is the phenotype of the mutant that is further downstream in the pathway. Overexpression of SEV alone causes too many R7 cells to develop, and a loss of function in Boss alone leads to no R7 cells. The double mutant leads to too many R7 cells developing. Thus one would place the Sev gene downstream of the Boss gene in the pathway for the development of the R7 cell. (Downstream means closer to the end of the pathway, or the output. Upstream means closer to the signal for the pathway, or the input.)*

b) Another gene involved in this developmental pathway is called *Sos*. You have isolated a gain of function mutation in this gene; you call this allele *Sos* *. You find that flies that are expressing *Sos* * in their eyes have high numbers of R7 cells.

What two strains would you make (given that you have gain-of-function and loss-of-function alleles of *Sev* and *Boss* available to you) to determine the position of *Sos* in the fly eye developmental pathway?

(8pts) *Boss* (-) *Sos* * (4 pts) and *Sev* (-) *Sos* * (4 pts) Credit for other potentially informative stocks; no credit for stocks with double over-expressing combinations. [Note: there are other options that distinguish among the 3 possibilities of order for the 3 genes, given that the order of Sev relative to Boss is already known. Example: *Boss* (-) *Sos* * and *Sev* (-) *Boss* (-) *Sos* ; others if *Sos* (-) is assumed to be available.]

*Explanation (not required for full credit):*

*To determine the order (Boss -> Sev -> Sos -> an R7 cell), you could have made: flies doubly mutant for *Sos* * and loss-of-function *Boss; AND flies doubly mutant for *Sos* * and loss-of-function *Sev* Note that you have to use double mutants that contain two single mutations that yield opposite phenotypes. That is, you couldn’t use a fly that was expressing *Sos* * and overexpressing SEV for instance, because both single mutant phenotypes = too many R7 cells.*

c) Assuming that *Sos* is downstream of *Sev* and *Boss* in the fly eye developmental pathway, what
phenotype would each of your strains in part (b) have?

(4 pts) Both strains would have a high number of R7 cells (4 pts). If suggested Sos(-) strain in part b, “no R7 cells” accepted.

d) By sequence and BLAST analysis, you determine that the Sev gene encodes a receptor tyrosine kinase (RTK). Given this information, propose molecular roles for the BOSS and SOS proteins.

(6 pts) BOSS is the ligand for SEV (2 pts; -1 pt if term ‘ligand’ is not used); SOS is a downstream effector (2 pts) such as … (G2 protein, transcription factor, phosphorylation target, in Ras pathway, etc.) (2 pts). Up to 2 compensatory points for general description of RTK activation and signaling pathway. If error in a-c resulted in assuming Sev->Boss->Sos, no deduction for description of BOSS as downstream player, but could receive full credit only if described role of ligand in compensatory description.