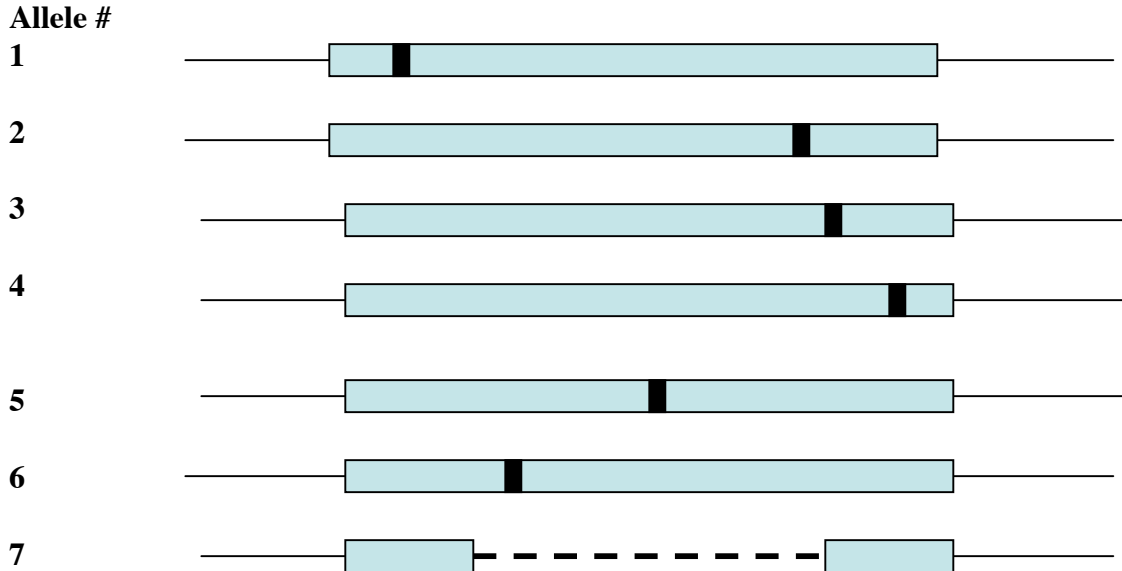


You Must Type Out Your Answers where your answers involve words.

1. List the three reasons given in class for “drug side effects”.
(This is a “regurgitation” question, of which there will be very few on an exam!)
2. ORFs. Refer to a Table of the Genetic Code (there is one in your text book. pg240)
 - A. Start with an “ATG” (the nearly-universal “start” codon). Not using the same codon twice, write out 30 consecutive “sense” codons. You have just generated a new “open reading frame”. Underline consecutive triplet bases, each encoding an amino acid, in this open reading frame.
 - B. Calculate the probability that, if you wrote down bases in random order (A,T,C,G) , that you would generate an open reading frame (i.e. one having no stop or non-sense codons”) that was 30 codons long.
 - C. Now delete the 4th base from the left. Underline the new reading frame. Is it “open”..encoding amino acids? Circle the first (if any) nonsense codon. By my calculation, about 4 in 5 of you should have sequences that end in a nonsense codon. Write down the amino acids that this new reading frame encodes. If your sequence did not result in an in-frame nonsense codon, go back to your original sequence, now add a base after the third base (the G of ATG), underline the new reading frame and circle the first (if any) nonsense codons.
 - D. Without considering your specific sequence, what is the probability that you will reach a stop codon by the 10th triplet? Hopefully you now understand why a “frameshift mutation” frequently eliminates a proteins function (as opposed to a missense mutation that may change one amino acid), either because of a nonsense codon or different amino acids.
 - E. If genome #1 is “AT rich’ compared to genome #2 (say an obscure yeast versus cow). That means that genome #1 has, say, 60% AT and 40% GC, while genome #2 is 50%AT and 50% GC. If a frameshift mutation occurs, is a nonsense codon more likely, less likely, or just as likely to occur in the new reading frame in a gene in the AT-rich genome #1 compared to genome#2. Explain briefly.
3. On the structure of mutational intermediates caused by base modification.
 - A. See Slide 23. Draw a G and a C base-paired, complete with their correct chemical structures and hydrogen bonding. Draw the ethylated G base-paired with T see in slide 23. The mechanisms of how ethylation causes a mutation by altering base pairing should now be clear.
 - B. Now look up and write down a second example that illustrates the chemistry of how a base pair mutation come about by base modification.
4. Fluctuation Test: Imagine you have 1000 bacterial haploid cultures that each started from 1 cell. The cells were Amp^R, and you screened for Amp^S in each culture. You allowed the cells to complete 10 cell divisions (to generate cultures of 10²⁴ cells each.)
 - A. Calculate the number of cultures with no mutants (the “0” class) and with >256 mutants if the mutation rate is 0.01 per cell division; if the mutation rate is 0.001 per cell division .
 - B. A fellow student comes to you and explains that he thinks he discovered a bacterial mutant that causes a higher frequency of mutation in the Amp^R gene. He bases this conclusion on the observation that, in mutant and wildtype cultures of 10⁶ cells he identified 200 and 20 mutants, respectively. What do you tell him?

5. Recombination Problem. You are given these 7 *Leu1*- alleles. The site of the mutation in 1-6 is shown in each allele by a vertical bar. For the “bar” mutations, the mutation is a missense. A deletion is shown for allele 7 by a dotted line....this represents the DNA that is missing from that allele.



Imagine that pairs of alleles are present in a diploid eucaryotic cell, so the cell is *Leu*⁻.

You can determine the frequencies of intragenic mitotic recombination by selecting for *Leu*⁺ progeny.

- Which single pair of alleles will give the highest rate of recombination, and why? Draw out the recombination reaction and the resulting diploid cells.
- Which single pair of alleles will give the lowest rate of recombination and why?
- If you have homozygous strains (in which both alleles are the same), which allele will most frequently revert to wildtype, and why?
- Which homozygous strain (in which both alleles are the same) will revert at the lowest frequency, and why?
- Will X-irradiation increase or decrease the recombination frequency? Explain why.

Answers to Problem Set #6, Due Oct 7.

1. A drug that inactivates a protein may have “side effects” because:
 - a. The protein effects more than one process
 - b. The protein acts directly in more than one process
 - c. The drug inactivates a domain that is shared with other proteins; the drug is essentially inactivating more than one protein.

2. A. Here is a sample sequence...I wrote down just 14 codons, you should have 30 down.

ATG AAA ACG AGG ACC TGG TGC TTT GGG CCC CTC GTG AGG GGC (ETC)

B. $(61/64)^{30} = 0.24$, so 24% of the time a random sequence of 30 codons will have an open reading frame. As you can see, the longer the sequence gets, the more improbable an open reading frame will be unless it encodes a bonafide protein! In yeast, ORFs of 100 amino acids are suspected of encoding proteins, which is reasonable because the probability of such a sequence existing by chance is ~1%.

C. Delete the 4th base, underline the new reading frame, and circle the first stop codon...

I actually went with slashes to show the frame..turned out to be easier in powerpoint..

ATG ~~X~~AA A|CG AGG A|CC TGG TGC T|TT GGG C|CC CTC G|TG AGG GGC

I did not write down the aminoacid sequence of my sequence, but you should see from yours that the specific amino acids are likely completely different after the deletion of a base compared to the sequence from your original ORF.

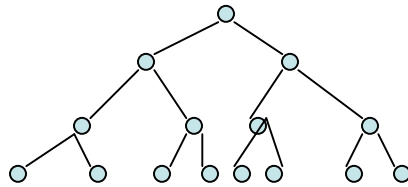
D. This is basically like part B....only it’s a “1 minus” problem set up. $(61/64)^{10}$; you have a 62% probability of not have a stop codon through codon 10, so that means you have a 38% probability of having a stop codon by codon 10.

E. If a genome is more AT rich, there is a higher probability of hitting a stop codon because 7 of 9 bases of the common stop codons shown in the Genetic table of the book (TGA, TAA, TAG) are A or T.

3. Draw out the structures from any source you could find. I found the base-pairing schemes in Molecular Biology of the Cell. I wanted you remind yourself of the geometry of base-pairing and how changing hydrogen-bond acceptors or donators alters base-pairing and generates a mismatch.

4. Ah yes. So what I should have said, and meant to say, was what are the jackpot cultures that contain at least 256 mutant cells. Asking for >256 mutant cells conceptually still gets us there, but it was clumsier. I was also assuming that only one mutational event occurs per culture. In general this is not a bad assumption with small cultures (1024 cells is a very small culture!!! Usually we deal with millions of cells, not thousands...). You might consider the case where two mutations occur...even at the extraordinarily high rate of 0.01 mutation per cell division, the frequency of two mutations will be 0.0001, or 1 in 10,000.

Anyway, here is the logic for at least 256 mutant cells. (For “greater than 256 cells” it has to be adjusted to be for at least 512 cells.) There was more information you needed than provided to get this completely, and I apologize for that. “It won’t happen again...”



In this pedigree, any culture with no mutants through these first 7 cell divisions will not generate jackpot cultures of at least 256 mutants. That’s because if any one of these cells mutates in the next cell division only 128 mutant cells will be generated. (If one of the first 7 cell divisions does generate a mutation, then I assumed that both product cells were mutant, as shown in the fluctuation figure in the book. This assumption that a cell division generates 2 mutant cells would not be known to you unless I told you that!) Anyway, if one a mutation did occur in one of these cell divisions, then at least 256 mutant cells would be formed in this culture.

Ok, with this, lets consider first the probability of the zero class which is easier to calculate:

Mutation rate of 0.01, its $.99^{1023} = 3$ in 100,000 cultures will be the zero clas, or 0.03/1000. Pretty infrequent, and that is because of the very high mutation rate (0.001 per cell division is unheard of.)

Mutational rate of 0.001, its $.999^{1023} \approx 36$, so 360 of 1000 cultures will be of the zero class. Note that a 10 fold increase in mutation rate gives a dramatic difference in the number of the zero class cultures.

The fraction of the jackpot class is trickier for the reasons stated above.

The probability of not having a mutational event in these first 7 division, if the mutation rate is 0.01 per cell divisio,n is $0.99^7 = .93$. So that means that 70/1000 cultures will have an event in those first 7 divisions.

(For 512 mutants per culture, use no mutational event in the first 3 cell divisions, and proceed.

If the mutational rate is 0.001, then the probability of not having a mutation in the first 7 divisions is $.999^7 = .993$, so 7 cultures in 1000 will be “jackpots”.

5. Some of this was not obvious, but hopefully it will be to you all very soon.

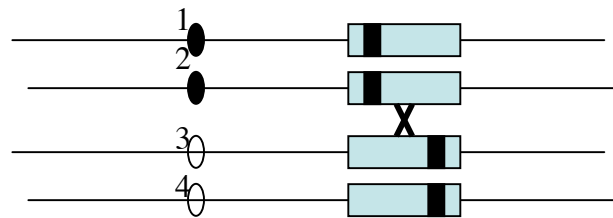
- a. Alleles 1 and 4 are furthest apart, and therefore will recombine to generate Leu+ progeny at the higher frequency. Remember, DNA damage causes breaks that occur spontaneously and randomly, and when they occur they can lead to crossovers. The further apart two alleles, the greater the chance for a DNA break and recombination between them.

See below for diagram.

- b. The lowest rate will be between 2 & 7; 3 & 7; 5 & 7, or 6 & 7. Each pair has no recombination.
- c. If the strains are homozygous, the only way they revert to wildtype is by mutation! And, alleles 1- 6 seem to have equal changes of such a reversion (they are all missense mutations and there is no reason to think one will revert any more frequently than another).
- d. Allele 7, the deletion, will revert at the lowest frequency because it is essentially impossible to replace all those nucleotides in their correct order with no template!!!! Note that alleles 1-6 require just a basepair change....like the his- to his+ reversion in the Ames Test.
- e. Xrays generates DNA breaks, and therefore X-rays increases recombination, and therefore X-rays leads to more recombination between any pair of alleles.

I can appreciate that some did not understand that recombination must have been to Leu+. That is all one can determine in this situation, right?

Here is one way to do it. Take a G2 cell with two sets of sister chromosomes of each of the two homologs.



If 1 and 3 cosegregate at mitosis, for example, one daughter cell looks like this:



You can also draw a recombination event between 2 G1 chromosomes, and that will also generate a cell in which one gene is wildtype and the second is a double mutant.