Welcome to the First Genetics Exam!

BIOL 213 GENETICS: Exam Sept 25, 2000

RULES OF THE GAME: This is an open book exam. It is an open notes exam. Needless to say, it is not an open people exam. This applies particularly to communication between people taking the exam at different times.

ANSWER SHEET: Put answers in the space provided, and turn in only the answer sheet plus any accompanying sheets. There's no need to turn in the questions. Please do not write in the margins. Leave them for us.

MEANING OF WORD-RESTRICTIONS: The less we have to read, the faster we can finish grading and get the exams back to you. Short answer questions will provide a suggested number of words. Please do your best to adhere to that number. If you need more space, provide your best short answer in the space, followed by a E, and only then amplify on the subject on the back of the answer sheet or on another sheet of paper. If your short answer is good enough, we can skip the explanations. If not, then we'll see what else you have to say.

WEIGHTS OF QUESTIONS: See the numbers in the parentheses for each question.

MULTIPLE CHOICE QUESTIONS: More than one answer may be correct, or none may be correct. Choose as many answers as you feel are correct. If you think none are correct, say so.

NEED A FACT? If there is some specific fact you think you need in order to answer a question, but you can't find it, then say so, make up the fact, and proceed.

NEED ANYTHING ELSE? A sheet of paper? A look at the textbook? A clue? Ask Brad or Jeff.

The Questions

1. (1) If you have neither received nor given aid regarding this exam, nor have you gained or given knowledge concerning a previous or future administration of this exam, then sign your name. Otherwise sign someone else's name.

2. (5) As you are taking this exam, an e-mail is speeding to your mailbox containing the web address of a questionnaire that solicits your views on the course thus far. It should take you 5 to 10 minutes to complete it. Do you agree to submit the questionnaire as soon as convenient but at least by 7:00 AM Friday, Sept 29?

Yes no

3. (2) The term "DNA", often encountered in the scientific literature, can be explained as follows:

- **A.** It is an abbreviation of deoxyribonucleic acid
- **B.** It is an abbreviation of dihydroneuraminic aldehyde
- **C.** It is an abbreviation of the National Dyslexic Association
- **D.** Originally pronounced "deh-nah", the word is a contraction of "denarius", a Roman coin, drawing on the metaphor of genes as the coins of inheritance.

4. (20) The following questions refer to your mystery sequence. You may either write your answers on the answer sheet or attach them as separate sheet.

4a. How many ORFs in each reading frame (remember that there are 6 such frames) can code for proteins of 100 amino acid residues or longer?

4b. What is the starting nucleotide number (A of ATG) and ending nucleotide number (last nucleotide of final codon, not the stop codon) for the largest ORF?

4c. Based on what you know so far, what may precede a biologically relevant ORF in bacteria? Does the largest ORF have it?

4d. Do any of your putative proteins appear to span a membrane? In 30 words or less, state the evidence behind your answer.

5. (8) Conclude as much as you can from the following results of an experiment in which artificial RNA was translated in an in vitro translation system.

CUCUCUCU. . .	ArgCysArgCys. . .
CUUCUUCUU. . .	CysLysAlaCysLysAla. . .

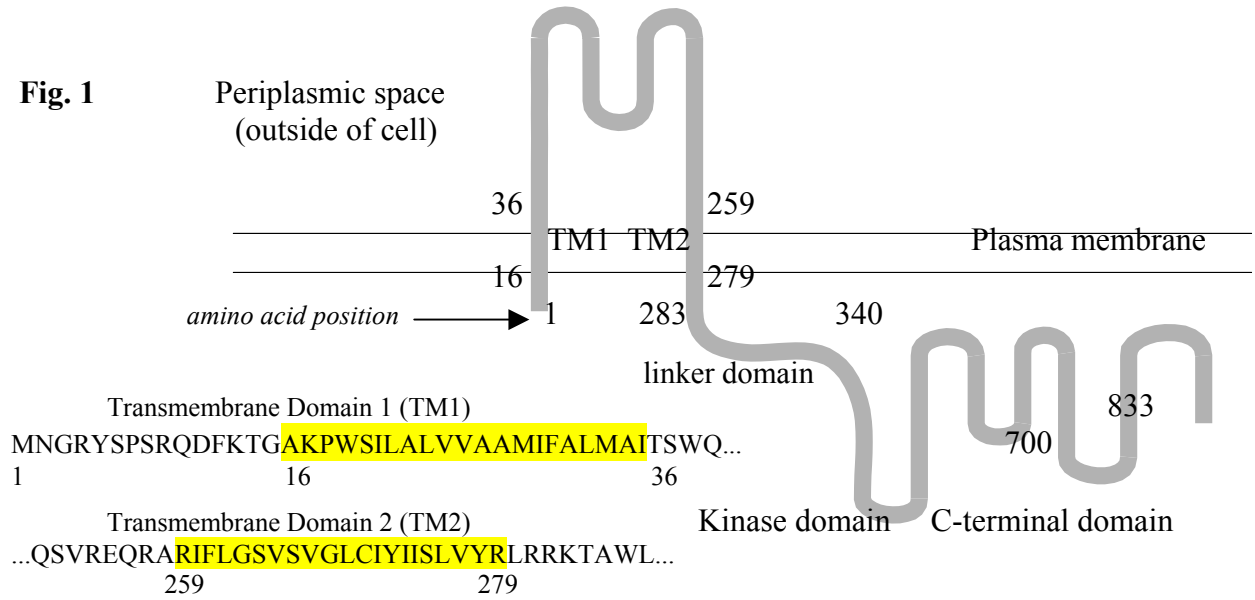
6. (15) A culture of the bacterium *Bacillus subtilis* was incubated with radioactive phosphate for twenty four hours, a long period of time, since the bacterium requires only 30 minutes to divide or (in a separate experiment) for twenty minutes. After the incubation, total DNA was isolated (and subjected to RNAse to destroy any RNA). At the same time, total RNA was isolated (and subjected to DNAse to destroy any DNA). The two samples (DNA and RNA) were hydrolyzed to produce their component bases, and the radioactive bases were analyzed, to give the results shown in Table 3.

6a. Explain in fewer than 10 words why the radioactive base composition of RNA resulting from the 24-hour incubation is so different from that of DNA.

6b. Explain in fewer than 20 words why the radioactive base composition of RNA resulting from the 20-minute incubation is so similar to that of DNA.

6c. Draw a picture of an autoradiogram of a gel with two lanes. The first lane contains RNA from the 24-hour incubation and the second contains RNA from the 20-minute incubation. An autoradiogram shows the position of radioactivity.

Fig. 1



We are becoming very familiar with *Agrobacterium tumefaciens*, a well known pathogen of plants (and animals too) that invades wounds. The *vir*A gene encodes an 833-amino acid protein (surprisingly called VIRA) that is involved in how the bacterium causes disease on plants. VIRA is a transmembrane protein that is activated by the binding of certain plant-derived molecules, such as acetosyringone (AS) and glucose (GLC). After binding, VIRA acts on another protein inside the cell to pass on the signal that a wounded plant is nearby. VIRA activity can be activated by high levels of AS or by low levels of AS plus GLC. With this knowledge under your belt, use the data in Problems 7 and 8 along with Fig. 1 above to determine how VIRA is activated by AS and GLC.

7. (9) Chang & Winans (1992), and Turk et al. (1994) made deletions in the *vir*A ene that removed coding region but maintained the same reading frame (see example below), transformed each mutated gene into a *A. tumefaciens* strain that lacked *vir*A, and then measured VIRA activity [none (-), very low (±), high (+), very high (++)].

region to be deleted

5'...ATGGGCTTCAAACGGATC...3' → 5'...ATGGGGATC...3'

translation: Met Gly Phe Lys Gly Ile

translation: Met Gly Ile

Portion of VIRA Protein Missing Due to Deletion in *vir*A Gene	VIRA Activity: no activators	high AS	low AS + GLC
no deletion (wt)	±	++	++
amino acids 40 - 240	±	++	±
amino acids 283 - 304	±	+	±
amino acids 340 - 700	-	-	-
amino acids 750 - 833	±	++	++

7a. In 10 words or less, where does AS bind to VIRA?

7b. In 10 words or less, where does GLC bind to VIRA?

7c. In 10 words or less, what part of VIRA most likely contains the active site for enzymatic activity?

8. (16 pts) Doty et al. (1996) randomly mutagenized the *vir* A gene with hydroxylamine to introduce single base substitutions. They introduced each mutant gene into a *A. tumefaciens* strain that lacks *vir*A, and then measured VIRA activity [none (-), very low (±), high (+), very high (++)].

Amino Acid Substitution in VIRA Protein Due to Mutation in *vir*A Gene	VIRA Activity: no activators	high AS	low AS + low GLU	
no mutation (wt)	±	++	++	
Ser20 to Phe	±	±	±	
Arg88 to Trp	±	++	±	
Asp139 to Asn	±	++	±	
Arg209 to Cys	±	++	±	
Glu210 to Lys	±	++	±	
Gly268 to Asp	±	±	±	
Thr284 to Met	±	±	±	

8a. What kind of change is Arg88 to Trp? (silent, conservative, charged hydrophilic to uncharged hydrophilic, hydrophilic to hydrophobic, etc.)

8b. What kind of change is Glu210 to Lys?

8c. What kind of change is Gly268 to Asp?

8d. In 20 words or less, does the data from Doty et al. change your view of here GLC binds to VIRA? If so, how?

8e. In 20 words or less, does the data from Doty et al. change your view of where AS binds to VIRA? If so, how?

8f. Given the kind of change involved in Gly268 to Asp, come up with an alternative explanation for the effect of this mutation that does not involve direct binding to AS and/or GLC (30 words or less).

9. (5 pts) In a particular bacterial species, G+C = 4(A+T) and A+G = C+T.

9a. What is the nucleotide composition of this organism?

9b. In 10 words or less, what assumption did you make to reach your answer in part a.?

10. (2 pts) As we already know, *Agrobacterium tumefaciens* has a G+C content of 64%.Given no other information, which one of the following enzymes will probably cut the *A. tumefaciens* genome the most number of times?

*Dra*I	5'-TTTAAA-3'
*Apa*I	5'-GGGCCC-3'
*Bam*HI	5'-GGATTC-3'
*Eco*RI	5'-GAATTC-3'

11. (8 pts) Aspartate transcarbamoylase (ATCase) is a multimeric enzyme of identical subunits that catalyzes the first committed step in the biosynthesis of pyrimidine nucleotides. The *pyr*B gene encodes the monomer subunit of ATCase. Use the information given below to figure out the effects of two different mutations in *pyr*B on ATCase structure and function.

11a. Mutation 1 was originally found in a strain of *E. coli* that completely lacked ATCase activity. The mutant *pyr*B$^-$ gene was cloned into a plasmid and then introduced into wildtype *E. coli*. In 30 words or less, deduce as much as you can from the table below about ATCase structure/function and the effect of mutation 1.

Strain	Amount of Subunit Protein	Quaternary Structure(s) Seen for Subunit Protein	ATCase Activity (Fraction of wt)
1. wildtype *pyr*B	60 units	hexamers only	1
2. *pyr*B$^-$ (mutation 1)	60	monomers only	0
3. wildtype pyrB on chromosome and pyrB$^-$ (mutation 1) on plasmid	60 (30 of each kind)	1/2 in monomers & 1/2 in hexamers	$^1/_2$

11b. Mutation 2 was originally found in a strain of *E. coli* that completely lacked ATCase activity. The mutant *pyr*B$^-$ gene was cloned into a plasmid and then introduced into wildtype *E. coli*. In 30 words or less, deduce as much as you can from the table below about ATCase structure/function and the effect of mutation 2.

Strain	Amount of Subunit Protein	Quaternary Structure(s) Seen for Subunit Protein	ATCase Activity (Fraction of wt)
1. wildtype *pyr*B	60 units	hexamers only	1
2. *pyr*B$^-$ (mutation 2)	60	hexamers only	0
3. wildtype pyrB on chromosome and pyrB$^-$ (mutation 2) on plasmid	60 (30 of each kind)	hexamers only	$^{57}/_{64}$

12. (3 pts) You are working in the Emerging Diseases Unit, the phat section of the Center for Disease Control in Atlanta. Several people attending the 75th Annual Clown Convention in Baltimore have come down with a mysterious malady where their noses are bulbous and red without makeup!! No one has cultured the pathogen yet, but some intact nucleic acid has been recovered from the noses of the victims that is not human in origin (don't ask me how we know that). You determine the nucleotide composition of the nucleic acid sample to be 20% A, 20% C, 30% G, and 30% T.

12a. Is the nucleic acid DNA or RNA, single-stranded or double stranded?

12b. Is the pathogen most likely a bacterium or a virus?

13. (6 pts) A linear piece of DNA has just finished replicating from an origin of replication approximately in the center of the molecule. Draw a simple picture of the finished replication products in a mutant *E. coli* strain that lacks the 5'-to-3' exonuclease function of DNA Polymerase I. Provide a legend to your drawing and be sure to indicate the directionality of each strand. (If you have to make any assumptions to answer this question, please state them.)

14. (15) ***E. coli***, like many other bacteria, are able to grow in the complete absence of any amino acids in the growth medium -- they can make all the amino acids themselves. Two strains of *E. coli*, JC158 (Lac$^+$) and AB3517 (Lac$^-$), were mutagenized with ultraviolet radiation and colonies are screened for those that are unable to grow on minimal medium unless the

medium is supplemented with the amino acid tryptophan. Three mutants were isolated in this way: Trp1, Trp2, and Trp3. The mutants have the following characteristics:

- When mutants Trp1 and Trp2 are spread on the appropriate indicator plates, they give rise to red colonies (owing to their Lac^+ phenotypes),
- When Trp3 is spread on the appropriate indicator plate, it gives cream-colored colonies.

Table 1: Characteristics of Trp^- mutants

Mutant	Parent	Lac phenotype	Chemical overproduced
Trp1	JC158	Lac^+	indole
Trp2	JC158	Lac^+	chorismate
Trp3	AB3517	Lac^-	anthranilate

- Chemical analysis of each mutant strain indicates that each excretes a characteristic chemical, as shown in Table 1.

Cultures of the three mutants are mixed together and plated on minimal medium plates (lacking tryptophan) that are supplemented with Lac indicator. The results are shown in Table 2.

Table 2: Results of mixing experiment

	Strain 1	Mixed with	Result
1	10^9 cells Trp1	---	No colonies
2	10^9 cells Trp2	---	No colonies
3	10^9 cells Trp3	---	No colonies
4	10^9 cells Trp1	100 cells Trp2	100 red colonies
5	10^9 cells Trp2	100 cells Trp1	No colonies
6	10^9 cells Trp1	100 cells Trp3	100 cream colonies
7	10^9 cells Trp3	100 cells Trp1	No colonies
8	10^9 cells Trp2	100 cells Trp3	No colonies
9	10^9 cells Trp3	100 cells Trp2	100 red colonies

14a. Which of the following are important factors in explaining the results:

A. DNA containing a *lac* gene is transferred between cells.
B. DNA containing a *trp* gene is transferred between cells.
C. An enzyme involved in lactose breakdown is transferred between cells.
D. An enzyme involved in tryptophan metabolism is transferred between cells.
E. Indole, chorismate, or anthranilate is transferred between cells.

14b. Using pictures and diagrams and as few words as possible, explain the results.

15. (6) The DNA below contains the start codon of the gene encoding a toxin from wild-type *Clostridium botulinum* (the nontemplate[1] strand of DNA is shown). The bacterium is mutagenized with proflavin, and the result is a single-base insertion at the position shown below:

GCTGAGCTATGA↓GATCCTTAGTGACTAACCGTACG

15a. Do you believe that the mutant toxin will be:

A. Completely functional
B. Partially functional
C. Completely nonfunctional

15b. What will be the last amino acid in the mutant protein?

[1] The template strand is the strand used by RNA polymerase to make RNA and so is complementary to it.

Table 3: Base composition of DNA and RNA[a]

	G	A	T or U	C
DNA				
20 min	15.8%	34.1%	35.0%	15.1%
24 hrs	15.6%	34.2%	34.8%	15.4%
RNA				
20 min	16.7%	33.2%	32.7%	17.4%
24 hrs	29.5%	28.1%	20.4%	22.0%

[a]Fraction of radioactivity in each of four bases after incubation with radioactive phosphate for indicated interval