BIO 304 Fall 2000 Exam II Name:_____
ID #:_____

1. Fill in the blank with the best answer from the provided word bank. (2 pts each)

incomplete dominance	conditional mutation	penetrance	expressivity	pleiotropy
Southern blotting	hybridization	epistasis	co-dominance	frameshift
nonsense mutation	translocation	syngamy	transformation	conjugation
missense mutation	clone	vector	YAC	BAC
electrophoresis	nitrocellulose	β-galactosidase	ampicillin	Prozac
transduction	silent mutation	prototroph	polymerase	transversion
reverse transcriptase	heterokaryon	bacteriophage	duplication	ligase
auxotroph	plasmid	probe	restriction enzyme	expression vector
lytic	transversion	episome	suppression	cDNA
lysogeny	complementation	stringency	RFLP analysis	polymorphism
Northern blotting	library	inversion	F factor	PCR

Nonsense mutation A nucleotide base change that results in a new codon specifying a stop codon.

Conditional mutation A mutation that causes a mutant phenotype only under restrictive conditions.

Co-dominance The condition when each allele produces a protein that can be detected in a heterozygote.

complementation The production of a wild-type phenotype when two recessive mutant alleles are united in the same cell.

Transduction Transfer of a bacterial gene by a bacteriophage.

F factor An *E. coli* plasmid that carries genes required for conjugation.

Translocation A chromosome rearrangement in which portions of two non-homologous chromosomes are exchanged.

Probe A labelled nucleic acid used to detect complementary sequences.

YAC The vector type which can carry the largest inserted DNA sequence.

BAC Prokaryotic vector type that can carry the largest inserted sequence.

Reverse transcriptase Enzyme necessary to make cDNA.

Restriction enzyme An enzyme that cleaves phosphodiester bonds.

Nitrocellulose Immobilizing membrane to which nucleic acids are covalently linked for blotting techniques.

Hybridization The process in which two complementary nucleic acid strands come together by hydrogen bonding to each other.

Ampicillin Drug often used to select for bacterial colonies that have been transformed with a plasmid.

2. Wild-type *Neurospora* can synthesize its own adenine from inorganic components in the growth medium. In a mutational analysis of the synthetic pathway for making adenine, ten adenine-requiring mutations (1 through 10) were recovered and heterokaryons were made in all pair-wise combinations, with the following results ("+" indicates that the heterokaryon grew in the absence of adenine):

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
1	-	-	+	+	+	+	-	+	+	+
2		-	+	+	+	+	-	+	+	+
3			-	+	-	+	+	-	+	+
4				-	+	-	+	+	-	-
5					-	+	+	-	+	+
6						-	+	+	-	-
7							-	+	+	+
8								-	+	+
9									-	-
10										-

a. How many genes are defined by these mutations? (5 pts.)

Three: complementation group 1 (1, 2, 7), group 2 (3, 5, 8), and group 3 (4, 6, 9, 10).

Mutants 1, 3, and 4 were tested for growth on the adenine-related compounds CAIR, AIR and SAICAR, with the following results ("+" means growth):

	adenine	CAIR	AIR	SAICAR
1	+	-	-	+
3	+	-	-	-
4	+	+	-	+

b. Explain these results. (5 pts.)

These mutations are in genes that encode enzymes necessary for different steps in the adenine biosynthetic pathway. The order of components in the pathway is:

AIR → CAIR → SAICAR → adenine

Mut 4 Mut 1 Mut 3

3. In the guinea pig, the following phenotypic pairs are each controlled by a single pair of alleles: *white* versus *black*, *short* versus *long*, *straight* versus *wavy*. Crosses were made between homozygous *white short straight* and homozygous *black long wavy* individuals, producing exclusively *black short wavy* F1 individuals. These F1 individuals were mated with *white long straight* animals, producing the following offspring:

Phenotype	#
white short wavy	46
white short straight	20
white long wavy	28
white long straight	6
black short wavy	6
black short straight	32
black long wavy	20
black long straight	42

a. Are the three allele pairs on the same chromosome? (2 pts.)

Yes

b. What is the apparent map distance between the *white/black* and *short/long* loci? (2 pts.)

c. Draw a map, including map distances, between these three loci. (4 pts.)

d. What is the coefficient of coincidence for apparent double crossovers in this interval? (1 pt.)

Obs./ Exp. = 0.06/(0.36 x 0.26) = 0.06/0.0936 = 0.64

e. What is the apparent interference value for double crossovers in this interval? (1 pt.)

I = 1- coefficient of coincidence = 1-0.64 = 0.36

4. A cross is made between an *E. coli* Hfr strain that is $a^+ b^+ d^+$ in genotype and an F^- strain that is $a^- b^- d^-$ in genotype. Interrupted-mating studies show that b^+ enters the recipient strain last. The b^+ recombinants were then tested for the presence of the a^+ and d^+ alleles. The following data were obtained:

$a^+ b^+ d^+$	326
$a^- b^+ d^+$	2
$a^+ b^+ d^-$	14
$a^- b^+ d^-$	58

a. What is the gene order? (4 pts.)

d^+ a^+ b^+

b. What is the frequency of recombination between genes *b* and *d*? (4 pts.)

76/ 400 =0.19 = 19 mu

* * * * * * * * * * * * * * *

5. A couple has a phenotypically normal son who, upon inspection of his sex chromosomes, is found to be XYY. In what parent could the nondisjunction event causing this chromosome constitution have occurred? (4 pts.)

father

At which meiotic division did this nondisjunction event occur? (4 pts.)

Meiosis II

* * * * * * * * * * * * * * *

6. The restriction endonuclease Bae II cleaves DNA at the following recognition sequence:

5' A C N N G T A Py C 3'
3' T G N N C A T Pu G 5'

(N means any base, Py means any pyrimidine, and Pu means any purine)

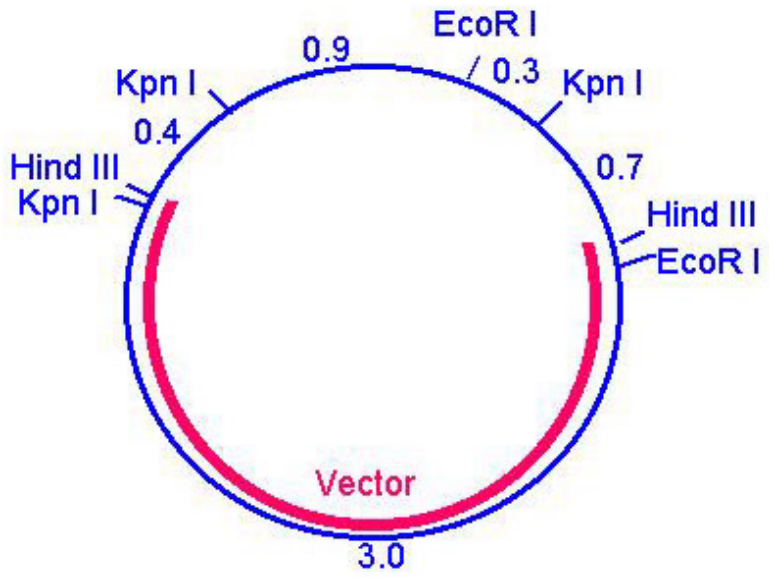
In an organism that has a random distribution of nucleotides, what would you expect to be the average size of restriction fragments from treatment of that genomic DNA with Bae II? Show your calculations. (5 pts)

The average fragment size is based on the frequency with which Bae II cuts DNA. That frequency is the product of the chance of having the right base at each position:
A=1/4 C=1/4 N=1 N=1 G=1/4 T=1/4 A=1/4 Py=2/4 C=1/4,
So the frequency is $(1/4)^6$ x ½ = 1/4096 x 0.5 = 1/8192 . If this is the frequency, then the average size restriction fragment must be 8192 bp long.

7. A plasmid library of human genomic DNA is constructed by cutting the DNA with Hind III and ligating into the Hind III site (AAGCTT) of the pBluescript II KS vector. You are given a single clone from this library ans asked to map this plasmid by restriction digestion. You perform the following digestions, separate fragments by agarose gel electrophoresis, and calculate the resulting fragment sizes. You come up with the following results.

Hind III	3.0, 2.3 kb
EcoR I	4.3, 1.0 kb
Kpn I	3.7, 1.2, 0.4 kb
Hind III + EcoR I	3.0, 1.3, 1.0 kb
Hind III + Kpn I	3.0, 1.2, 0.7, 0.4 kb
Kpn I + EcoR I	3.0, 0.9, 0.7, 0.4, 0.3 kb

a. Based on these data and the map of pBluescript II KS, construct a restriction map of the plasmid. Be sure to indicate sizes between restriction enzyme sites. (**Hint**: Remember that restriction fragments of less than 100 bp will not be visible by agarose gel electrophoresis.). (8 pts)



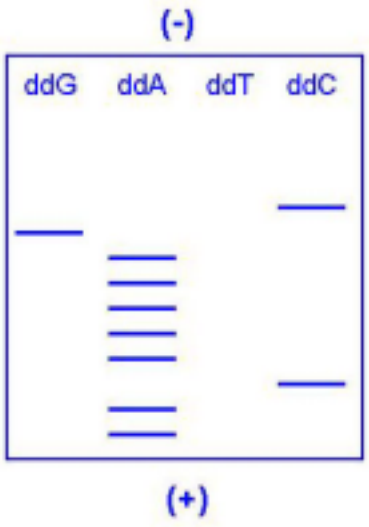
b. Indicate on your map the location of fragments that come from the pBluescript II KS vector. (2 pts.)

8. You have been given a copy of the restriction map and sequence of the polylinker for the plasmid vector pBluescript II KS. You perform Sanger (chain termination) sequencing on this plasmid to confirm the reported sequence. You use labelled T3 primer, as indicated on the map.

a. Name all the components you will need to put into test tubes to perform the sequencing reactions. (5 pts)

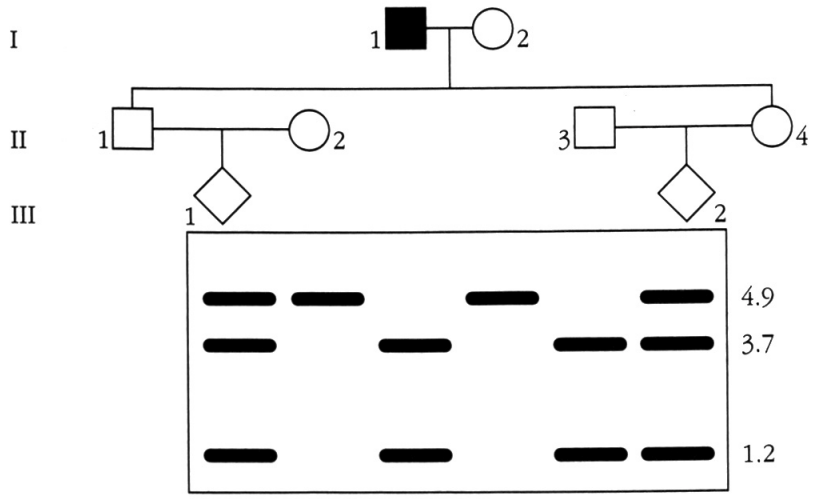
template, primer, dNTPs, ddNTP, and polymerase
(+1 extra credit for Mg^{++})

b. You perform electrophoresis on your sequencing reactions and determine that the reported sequence is completely accurate. Draw a diagram of what the gel looked like for the sequence of the DNA from the first ten nucleotides. Clearly label each lane of the gel. Show the polarity of the electrodes (pos and neg) relative to the orientation of your diagram. (8 pts)



* * * * * * * * * * * * * * *

9. The following pedigree comes from a family with a history of a dominant form of Parkinson disease. The symptoms of this disease typically occur later in life (after age 40), therefore cannot be diagnosed by physical examination during early or middle age. However, in this family, the father (I-1) was afflicted with Parkinson disease at a very young age. This is often a sign that the individual is homozygous for a disease allele. The mother has no Parkinson symptoms, but their children are still too young to be diagnosed. Both children are about to have children of their own and would like to know whether these grandchildren are at risk for the disease. Analysis of a RFLP that has been closely linked to this disease allele was performed on the individuals indicated by the blot shown below the pedigree. The RFLP result is diagrammed directly below the corresponding individual from the pedigree. Based on previous experience, it is known that **this RFLP is one map unit away from the Parkinson disease gene**, therefore it is a good, but not perfect predictor of inheritance of this allele. Based on this knowledge and the provided figure, answer the following questions.



a. For each of the two fetuses (III-1 and III-2), calculate the chance that they will develop Parkinson disease at some time during their life. Show your work and your reasoning. (8 pts)

In this case, the grandfather (I-1) is homozygous for the Parkinson allele and homozygous for the RFLP that maps near it. The grandmother is homozygous for a different RFLP morph and is not afflicted, so must be homozygous wild-type for Parkinson. Both the grandchildren are heterozygous for the polymorphism, but they inherited the alleles from different families. For III-1, the 4.9 morph came from the mother, who is presumably not at risk for Parkinson. That means that the 3.7/1.2 morph came from the father (II-1). That allele was inherited from the grandfather and is closely linked to the Parkinson mutation. There is a small chance (1 map unit, therefore 1 %) that the 3.7/1.2 RFLP morph was inherited separate from the Parkinson allele. We must therefore conclude that there is a 99% chance that grandchild III-1 will develop Parkinson disease. Grandchild III-2 inherited the 3.7/1.2 morph from the unaffected parent (II-3), therefore received the 4.9 morph from the parent carrying the Parkinson allele (II-4). Because the Parkinson allele is associated with the 3.7/1.2 morph in the grandfather, the only chance that grandchild (III-2) carries the Parkinson mutation is if there was recombination between the Parkinson gene and the RFLP in the mother (II-4). Again, the distance between these is 1 map unit, therefore there is only a 1% chance that III-2 will carry the Parkinson mutation.

b. The two children of the affected man (II-1 and II-4) were not tested themselves because they did not want to know whether they would develop Parkinson disease later in life. Based on the other data shown, which of the restriction fragments would you find if you had typed the son (II-1)? What is the chance that this man will develop Parkinson symptoms later in life? (5 pts)

II-1 will be heterozygous for the morphs, so will have all three fragments. Because his father (I-1) is homozygous for Parkinson, he will be heterozygous and has a 100% chance of developing the disease.