Name: _____

7.016 EXAM 2
October 24, 2018

TA:_____ Recitation: _____

The Exam starts at 10:05 am and ends at 10:55 am.

Write your name on this page and your initials on all the other pages in the space provided. This exam has **6** pages including the coversheet. Check that you have all the pages **1-6**.

Only answers on the **FRONT** of each page will be graded. You may use the backs of the pages, but only as scratch paper.

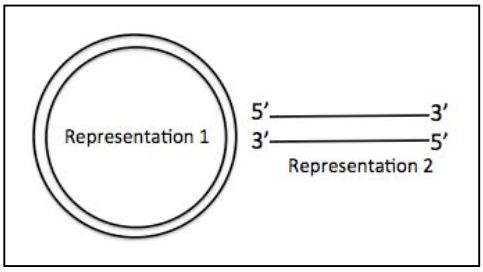
Questions	Points	Score
1	5	
2	22	
3	28	
4	20	
5	16	
6	9	
Extra Credit	3	
TOTAL	100	

Initials: _____

Question 1 (5 points)
You label the DNA of a human cell using BrdU (a nonradioactive thymidine or "T" analog). You remove the nucleus of this cell. You see that the cell still has some BrdU labeled DNA.

a) Which **organelle** in this cell has the remaining BrdU labeled DNA? _____

b) Which of the following represents the structure of the remaining BrdU labeled DNA and **why**: **Representation 1 or Representation 2**?



Question 2 (22 points)
In a fly, **Gene A** (alleles A and a) regulates **antennae length** and **Gene B** (alleles B and b) regulates **body segments**. Both genes are located on the same autosome.

a) You mate the P1 and P2 flies to get the following F1 flies (**Long antennae/ segmented**). Using uppercase letters for the alleles conferring the dominant phenotypes and lowercase letters for the alleles conferring the recessive phenotype, give the genotypes of…

i. True breeding **P1 fly** (**long antennae/ non-segmented): _____**
ii. True breeding **P2 fly (short antennae/ segmented): _____**
iii. F1 flies (**long antennae/ segmented): _____**

b) Hypothetically assume that the two genes are **6cM** apart. Based on this assumption, fill in the table below for **100 F2 flies** that are produced by mating an **F1 fly with another fly (Genotype: aabb)**.

Genotypes?	**Corresponding phenotype…**		**Corresponding numbers?**	**Is this a Recombinant OR parental class?**
	Antennae length?	**Body segments?**		

c) However, when you actually **mate two F1 flies** and look at the ratio of the resulting **100 F2** flies, you realize that the two genes are **completely linked**.

i. Give the **genotype** and the **corresponding ratio** of F2 flies**: _____**

ii. Give the **phenotype** and the **corresponding ratio** of F2 flies**: _____**

Initials: _____

Question 3 (28 points)

As a budding genetics expert, you mate two **true breeding flies** and obtain F1 flies that are heterozygous for Genes A, B and D on an autosome. You subject F1 to a **test cross** and obtain the following **F2 flies.**

F2 genotypes	Numbers
bDA /bda	390
Bda / bda	410
	75
BDa /bda	65
BdA /bda	30
	20
BDA /bda	6
bda /bda	4
TOTAL	**1000**

a) Give the genotype of each **of the following flies**.

i. **True-breeding** Parental fly 1: _____

ii. **True-breeding** Parental fly 2: _____

iii. **F1 fly:** _____

iv. The fly to which the F1 fly was mated in a test cross:

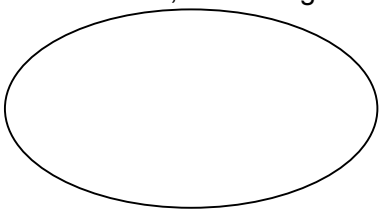
_____

b) In the table, fill in the missing genotypes in the **two shaded boxes**.

c) Calculate the map distance (in cM) between each gene pair combination: **B-D, A-B, A-D.** ***Note:*** *Consider ALL recombination events where needed while calculating the map distance.*

d) For an F1 cell undergoing meiosis, draw the arrangements of the alleles of B, D and A genes…

i. On the **duplicated homologs** during **Meiosis-I.**



F1 cell in Meiosis-I

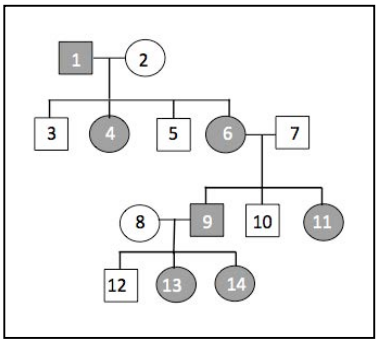
ii. In the products of **ALL single crossing over (SCO)** and **double crossing over (DCO)** events between B, D and A genes. ***Note:*** *For each product of meiosis, you should specify the genes pair combination between which the crossing over took place.*

Products of SCO	**Products of DCO**

Initials: _____

Question 4 (20 points)

The following pedigree shows the mode of inheritance of a RARE disease that is associated with a mutation in Gene A. ***Note:*** *Individuals 7 and 8 do not have any disease-associated allele of Gene A.*



a) Give **one of the possible modes** of inheritance of this disease: X-linked dominant/ autosomal dominant/ autosomal recessive/ X-linked recessive.

b) Give the possible genotype(s) of **Individual 5 for the mode of inheritance** that you selected in part (a) using "A" or "X^A" for the allele that confers the dominant phenotype and "a" or "X^a" for the allele that confers the recessive phenotype: _____

c) Individual 11 has a son with a normal, healthy male. What is the probability that their son will be **affected**? _____

d) You suspect that the enhancer sequence of Gene A may be mutated in patients. You therefore decide to characterize it. Which library would you use to identify the bacterial clone carrying the enhancer sequence specific to Gene A and **why**: **genomic or cDNA** library?

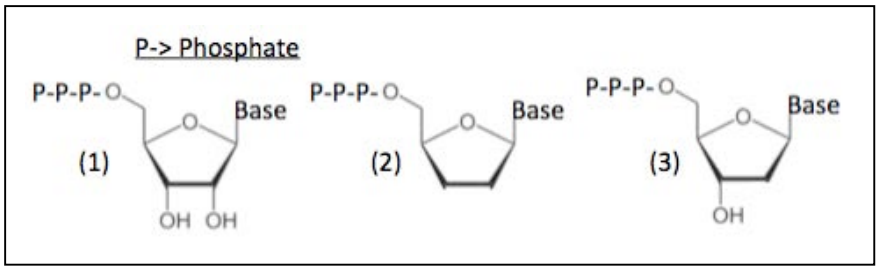
e) You isolate the plasmid that has the "enhancer sequence insert" and PCR amplify it. Give the sequence of the 6-bases long primer to make the….

```
5'GCTAGG_____CGTCAA3' TOP
3'CGATCC_____GCAGTT5' BOTTOM
         Enhancer
```

I. **The Top strand:** 5'_____3'

II. **The Bottom strand:** 5'_____3'

f) You sequence the PCR amplified enhancer sequence. Which of the following nucleotides is used in Sanger DNA sequencing but **NOT** in PCR and **why**?



Initials: _____

Question 5 (16 points)

You would like to understand the localization of the protein encoded by Gene A in a patient's cells. You therefore ligate the cDNA sequence corresponding to the C-terminus of Gene A with the cDNA sequence corresponding to the N- terminus of GFP gene to make a **Gene A-GFP fusion cDNA** that encodes the **Protein A-GFP fusion protein**.

The following is the partial cDNA sequence encoding the C- terminus of Gene A. ***Note:*** *The DNA corresponding to the stop codon is bold and underlined. The sequence specifically recognized by each restriction enzyme is shown in gray. Each codon is separated from the next by a space.*

				1					3				5		
A:	5′	AAA	ATT	CTG	CAG	AAT	ACA	ATT	CCG	CTG	CAG	**TAG**	TTT	GAA	TTC	ATC 3′
	3′	TTT	TAA	GAC	GTC	TTA	TGT	TAA	GGC	GAC	GTC	ATC	AAA	CTT	AAG	TAG 5′

The following is the partial cDNA sequence encoding the N-terminus of GFP gene. ***Note:*** *The DNA corresponding to the start codon is bold and underlined. The recognition sequence for each restriction enzyme is shown in gray. Each codon is separated from the next by a space.*

				2			5			4							
GFP:	5′	ATG	TGC	AGG	GCG	GAA	TTC	GGG	TTG	CAA	**ATG**	CCA	CTC	GAG	GAA	TTC…3′	
	3′	TAC	ACG	TCC	CGC	CTT	AAG	CCC	AAC	GTT	TAC	GGT	GAG	CTC	CTT	AAG…5′	

The recognition sequences and the cleavage sites (*indicated by /)* for each enzyme are given below.

1	***2***	***3***	***4***	***5***
5′C/TGCA G3′ 3′G ACGT/C5′	5′G/TGCA G3′ 3′C ACGT/C5′	5′C TGCA/G3′ 3′G/ACGT C5′	5′T TGCA/A3′ 3′A/ACGT T5′	5′G/AATT C3′ 3′C TTAA/G5′

a) Which restriction enzymes pair would you use to make the Gene A-GFP fusion cDNA that can be cloned in a plasmid and expressed in bacteria? **Explain** why you selected this pair and **NOT** the others.

Pair A: I & 2 **Pair B:** 3 & 4 **Pair C:** 5 & 5

b) Give the 6-base pair sequence at the point of ligation of the C-terminus of Gene A with the N-terminus of the *GFP* gene.

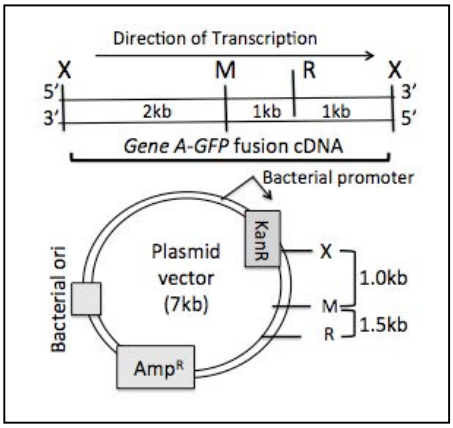
5'_____3'

3'_____5'

Initials: _____

Question 5 continued

You clone the Protein A-GFP fusion gene into the following plasmid and use it to transform the bacteria.
Note: *Both the Protein A-GFP fusion cDNA and the plasmid have the sequence for restriction enzymes X, M & R. The plasmid also has the ampicillin resistance (Amp^R) and kanamycin resistance (Kan^R) genes.*



c) How would you **select and screen** for bacterial colonies that have the recombinant plasmid?

d) You analyze a bacterial colony that has the recombinant plasmid with the Protein A-GFP insert. You want to determine the orientation of the Protein A-GFP insert within the recombinant plasmids. You isolate the recombinant plasmid from the bacterial colony, cut it with a restriction enzyme and resolve the resulting DNA fragments on a DNA gel.

Which restriction enzyme would you use to determine the orientation of the insert**: X/ M/ R**? **Explain,** why you selected this restriction enzyme. ***Note:*** *There is only one correct option.*

Question 6 (9 points)

a) Outline genetic crosses you would perform using flies to screen for **dominant mutations** in a gene that would result in a desired phenotype. ***Note:*** *Be sure to indicate which flies you would mutagenize, which generation you would examine for the phenotype and how many flies at a particular generation would be available for you to see the phenotype.*

b) If you discovered a fly gene, what would you name it and what biological process would be affected? **(Extra credit question: 3 points)**

7.016 Introductory Biology
Fall 2018