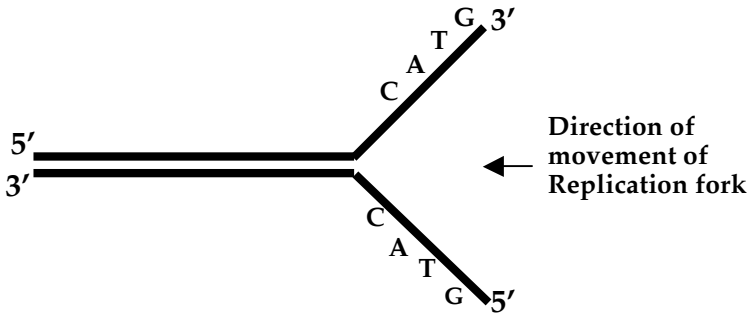
Name: _____

Question 1 (20 points)

a) In the table below, name the **sub-cellular location or organelle(s)** of the **eukaryotic cell** that will fluoresce when the following macromolecules are tagged with a fluorescent dye.

Macromolecules tagged with fluorescent dye	**Sub-cellular location or organelle(s) of cell that will fluoresce**
Proteins that add carbohydrates or lipids to the newly synthesized proteins	
Proteins that are a part of **functional** ribosomes	
DNA	

b) Shown below is a segment of replicating DNA in the epidermal cells of the mice.



i. On the schematic, draw the **elongating DNA strands** and **label their 5′ and 3′ ends**.

ii. To which strand **(***choose from top, bottom or both*) can primer **5′CATG3′** bind during replication?

iii. Which strand (*choose from top or bottom***)** is the template for **discontinuous** (**lagging) strand** synthesis?

iv. **Circle** the protein/ enzyme that relieves a replicating segment of DNA from super-coiling.

Helicases **Topoisomerase** **Primase** **Single stranded DNA binding proteins (SSDBP)**

c) You treat mouse epidermal cells in a plate with the drug, TAT-2. You observe that TAT-2 treated cells show reduced shortening of their chromosomes following each cell division and survive longer than the untreated cells.

i. Name the replication enzyme that serves as the target of TAT-2 and state whether TAT-2 *activates* or *inhibits* the function of this enzyme.

ii. Why does reduced shortening of chromosomes following each cell division promote long- term survival of a cell?

d) In a separate experiment, you irradiate the mouse epidermal cells, growing on a plate, with UV light. This treatment results in the formation of a **thymine dimer** (*a covalently joined pair of T-bases shown as bold and underlined*) as shown in the DNA segment below.

5′ ——— CT**TT**GCA ——— 3′
3′ ——— GAAACGT ——— 5′

Circle the process(s) (*choose from proofreading, excision repair or mismatch repair*) that will remove the thymine dimer and name the specific replication enzyme(s) that will **fill in and seal the gap** left after the removal of thymine dimer.

Enzyme that fills in the gap: _____ **Enzyme that seals the gap:** _____

Name: _____

Question 2 (18 points)

The following is the DNA sequence for the transcription initiation region of **Gene A that is expressed in epidermal cells** of mice. ***Note:*** *Part of the* ***promoter region*** *is boxed. Transcription* ***begins at and includes*** *the bold and underlined* ***A/T*** *base pair.*

```
5'----TGGACTGCTA  TAATAGCAGG  GCTGCCGAAT  GTGCTGCCAT  ACGGCCATGG TTCTTAAAGT----3'
3'----ACCTGACGAT  ATTATCGTCC  CGACGGCTTA  CACGACGGTA  TGCCGGTACC AAGAATTTCA----5'
```

a) Which DNA strand (*choose from top or bottom*) serves as the **template strand** for transcription?

b) Fill in the **first 6 nucleotides** of the **primary/ nascent mRNA** transcribed from Gene A.

5' ___ ___ ___ ___ ___ ___ 3'

c) Fill in the **first four amino acids** of Protein A encoded by Gene A. ***Note:*** *A codon chart is provided on the last page.* ***You can detach the last page.***

N _____ _____ _____ _____ C

d) The last 5 **amino acids (amino acid^{105}- amino acid^{109}) at the C- terminus** of wild-type Protein A are indicated below. Each of these amino acids is critical for the proper folding of this protein.

N - pro^{105}-asn^{106}-ser^{107}-met^{108}-leu^{109}-C

The DNA sequence encoding the **above 5 amino acids** is included within the sequence below.

Wild-type

```
5'-AACCGAATTCCATGTTATAGC-3'
3'-TTGGCTTAAGGTACAATATCG-5'
```

You isolate and sequence the following two different mutant alleles of **Gene A** that encode the above 5 amino acids. Each mutant allele is due to a **point mutation** that is **bold** and **underlined**. Which of these mutants will **ALTER** the folding of Protein A (*Choose from mutant 1 or mutant 2*)?

Mutant 1

```
5'-AACCAAATTCCATGTTATAGC-3'
3'-TTGGTTTAAGGTACAATATCG-5'
```

Mutant 2

```
5'-AACCGTATTCCATGTTATAGC-3'
3'-TTGGCATAAGGTACAATATCG-5'
```

Explain, in terms of the change in the reading frame and/ or amino acid sequence, why you selected this mutant and **NOT** the other.

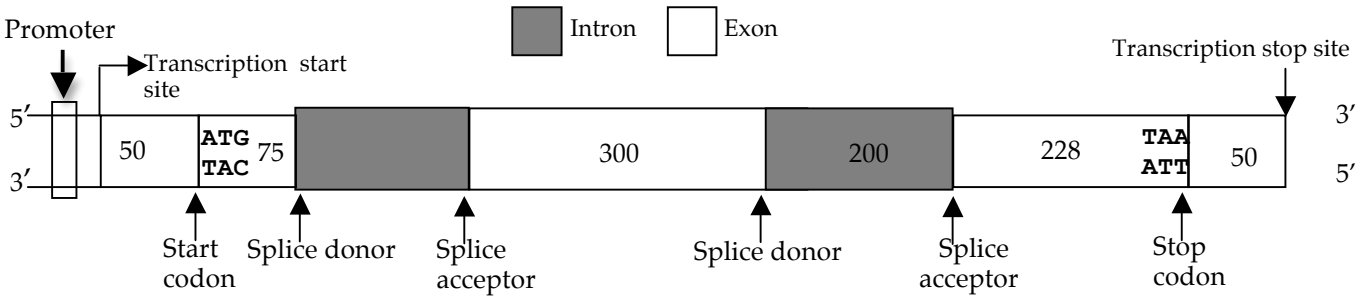
e) You identify a disease of epidermal cells in mice in which **Gene A is not transcribed**. Further analyses reveals that the sequence of Gene A in affected and normal mice is the SAME. **Circle** the options, from the choices below that could explain why Gene A is **NOT** transcribed in the epidermal cells of the affected mice. In the epidermal cells of affected mice…

1. Mature mRNA corresponding to Gene A lacks the 5 methyl Cap and 3' Poly A tail
2. DNA around the promoter region of Gene A is methylated
3. Epidermal cells of affected mice lack the transcription factors (TF) associated with Gene A.
4. The Ribosome binding site in mature mRNA transcript corresponding to Gene A is mutated
5. The 3' untranslated region (3'UTRs) of mature mRNA corresponding to Gene A is mutated
6. Histones close to the Gene A are acetylated

Name: _____

Question 3 (20 points)

Shown below is the schematic of Gene A. The numbers within the boxes indicate the length (in base pairs) of each region. The DNA sequence corresponding to the translational start and the stop codons is shown and the splice donor and splice acceptor sites are indicated.



a) You observe that Gene A is transcribed both in epidermal and muscle cells to produce a nascent / primary mRNA transcript. This mRNA directs the synthesis of two different proteins in these two different cell types.

- *In the muscle cells Gene A encodes a **protein** (100 amino acids long) that functions as a **nuclear protein (TF-1)***
- *In epidermal cells, Gene A encodes a **protein** (200 amino acids long) that functions as a cell membrane protein.*

Could Gene A direct the synthesis of **two different proteins** due to the…

Difference in….	**Explain** why you selected this option
Splicing (*Yes/ No*)?	
Protein processing (*Yes/ No*)?	
Promoter sequence (*Yes/ No*)?	

b) You want to study **another nuclear protein, TF-2** in mouse muscle cells. You identify a mutant cell line, which shows a **cytosolic location of TF-2** in muscle cells.

i. Name a stretch of amino acid sequence that the TF-2 in mutant muscle cell line lacks.

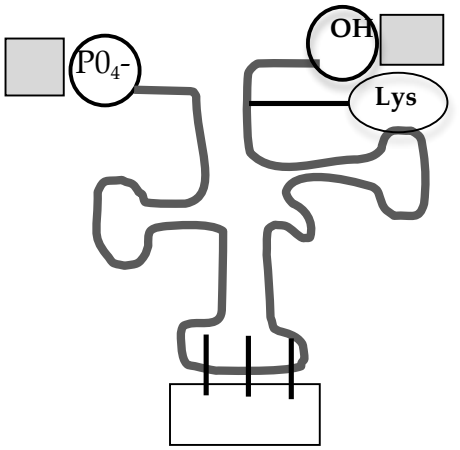
ii. In the wild-type muscle cells, if this stretch of amino acid sequence is located at the N-terminus of TF-2, where in the mature mRNA transcript (*choose from the 5′ end or the 3′ end*) would the corresponding base sequence be?

iii. The proteasome is a multi-protein complex that degrades any misfolded protein in a cell. How does the proteasome recognize which proteins in the cell are misfolded?

Name: _____

Question 3 continued

c) The lys^{60} (encoded by **5′AAG3′ codon**) of TF-2 is critical for its binding with its target sequence. The following is a schematic of tRNA specific for lys^{60} (encoded by **5′AAG3′ codon**).



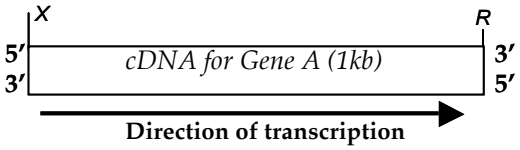
i. In the **blank box,** write the **anti- codon** that base pairs with the 5′AAG3′ codon for Lys**.**

ii. Label the 5' and 3' ends of the tRNA by filling in the shaded boxes.

d) Is the tRNA in the schematic *charged* or *uncharged*? **Explain** why you selected this option.

Question 4 (24 points)

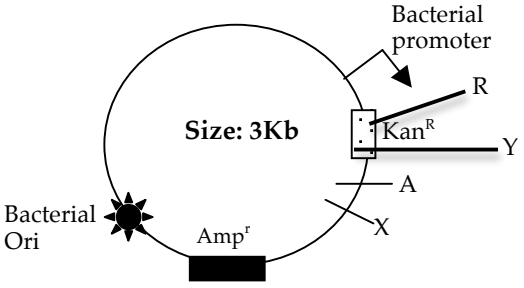
You decide to further characterize the TF-1 protein encoded by Gene A in muscle cells of mice. You adopt the following strategy to get a large amount of TF-1 protein for characterization. You make the cDNA using the mRNA derived from the wild- type allele of Gene A and by adding oligo- dT primers. The Gene A cDNA has the recognition sites for restriction enzymes X and R as shown below.



a) From the choices below, **circle** the sequences that are a part of the Gene A but are NOT contained in the corresponding cDNA.

Promoter **Exons** **Introns** **Enhancers** **3′UTR**

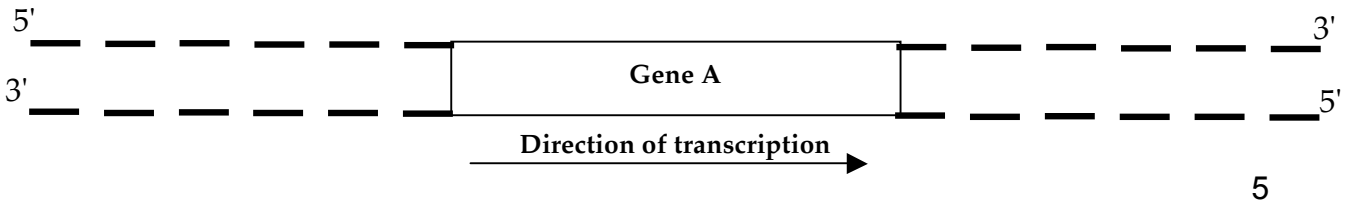
b) You want to clone the cDNA for Gene A into the following plasmid that has recognition sites for restriction enzyme Y, R, X and A as shown. ***Note****: A vertical line (|) represents the cutting site for each restriction enzyme.*



X	**A**	**R**	**Y**
5′G/TCGAC3′	5′GAATT/C3′	5′CAATT/G3′	5′C/TCGAG3′
3′CAGCT/G5′	3′C/TTAAG5′	3′G/TTAAC5′	3′GAGCT/C5′

i. Which enzyme(s) (*choose from X, A, R or Y*) would you use to cut the plasmid that would allow **directional cloning and expression** of Gene A from the bacterial promoter?

ii. Write the resulting **6- base pair sequence** at the **two points of ligation** after Gene A inserts into the plasmid.



Name: _____

Question 4 continued

c) You then plan to amplify the recombinant plasmid in *E. coli* bacterial cells. You transform the *E. coli* with the ligation mix and plate them on a master plate (*growth medium with no antibiotics*). You then replica- plate the colonies on **plate 1** (*growth medium + ampicillin*) and **plate 2** (*growth medium containing both ampicillin and kanamycin*). You obtain the following colonies.

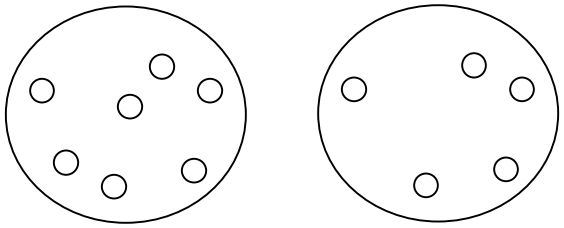


Plate 1 **Plate 2**

i. **Circle** the plate (*1 or 2*) in the schematic that contains bacterial colonies that have the **recombinant plasmid.**

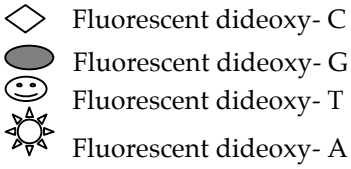
ii. In the plate that you circled, **fill in/ color** the colonies that contain the **recombinant plasmid**. **Explain** why you selected these colonies.

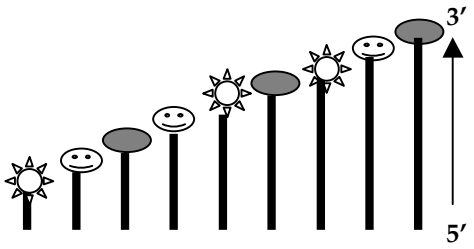
d) Another group is competing with you. Although they have the bacterial clone that contains the recombinant plasmid with Gene A in correct orientation they cannot express Gene A in bacteria. To understand what the issue is they decide to PCR amplify and sequence Gene A isolated from their bacterial clone (**mutant**) and compare it with the sequence of Gene A that you published (**wild- type**). Shown below is the sequence flanking the **mutant allele** of Gene A.

They have the following primers for amplifying Gene A by PCR reaction. **Circle** the **primers** that they would use to PCR amplify Gene A.

#1: 5′GAAATC3′ **#2: 5′TTCAGG3′** **#3: 5′CTTTAG3′** **#4: 5′AAGTCC3′**

e) Sequencing results show that the mutant version has **one point mutation** compared to the wild- type version of Gene A. Shown below is a portion of the fluorescence dideoxy- sequencing gel that gives the sequence of the **mRNA like strand/ non-template strand** of the DNA that corresponds to amino acids 5-7.





i. Write the sequence of the **mRNA** that corresponds to amino acids 5-7 of the mutant allele of Gene A.

5′----------3′

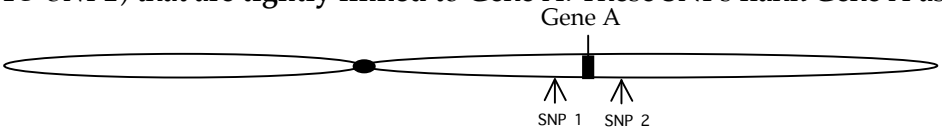
ii. If the wild –type allele has the amino acids **N- met^5-trp^6-met^7-C, circle** the base in the sequence that you gave in part (i) that has undergone point mutation in the mutant allele. ***Note:*** *Codon chart is provided on the last page.* ***You can detach the last page.***

iii. **Circle** the type of mutation that you see in the mutant allele from the choices below (*choose from silent, missense, nonsense or frameshift*).

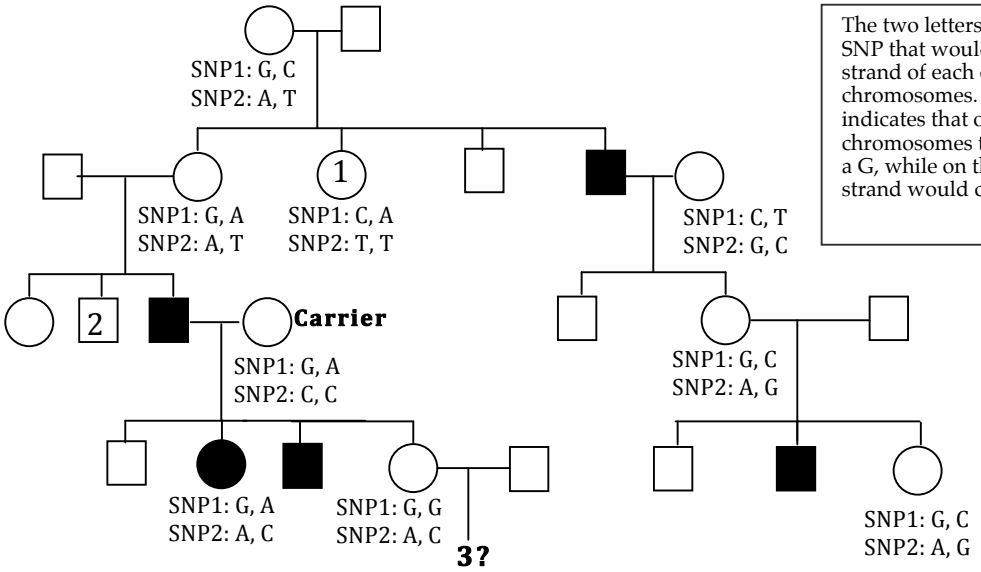
Name: _____

Question 5 (18 points)

You are studying a genetic disorder that is associated with a mutation at the Gene A locus. You identify two SNPs (*SNP1& SNP2*) that are **tightly linked** to Gene A. These SNPs flank Gene A as shown below.



Please note: *All the individuals with the disease phenotype are shaded. People marrying into the family have only the wild-type alleles of Gene A unless* ***indicated as a carrier****. Also listed are the alleles of SNP1 and SNP2 for some individuals. Assume* ***complete penetrance of the disease phenotype*** *and* ***NO Recombination*** *between SNP1, SNP2 and Gene A.*



The two letters identify the alleles of the SNP that would be found on the "top" strand of each of the two homologous chromosomes. For example, "SNP 1: G,A" indicates that on one of the homologous chromosomes the top strand would contain a G, while on the other chromosome the top strand would contain an A.

a) Give the most likely **mode of inheritance** of this disease.

b) Give the **genotype(s)** of **Individual 1** at Gene A locus based on her SNP1 & SNP2. ***Note:*** *Use the letter "A" or X^A to represent the allele associated with the dominant phenotype and 'a" or X^a to represent the allele associated with the recessive phenotype.*

c) Give the alleles of SNP1 & SNP2 that are tightly linked with the allele of Gene A in **Individual 2**.

SNP1: **SNP2:**

d) If **Individual #3 is a male**, what is the probability that he will be affected?

e) The above disease is also observed in mice. You mate an affected female with an affected male to get a fertilized ovum. You then successfully introduce a wild- type allele of Gene A into the fertilized ovum and implant it into a pseudo-pregnant female mouse. You observe that it develops into a **newborn male**.

i. What would be the genotype of **all** somatic cell-types in this newborn?

ii. Will the introduced gene be passed on to the subsequent generations by the transgenic mouse (*choose from yes, no or may be*)? **Explain**.

f) Why are two SNPs flanking a Gene regarded as better markers to predict its mode of inheritance compared to only one SNP located at one end of the Gene?

Name: _____

Codon Chart: You can detach this page

	U	**C**	**A**	**G**
U	UUU Phe (F) UUC " UUA Leu (L) UUG "	UCU Ser (S) UCC " UCA " UCG "	UAU Tyr (Y) UAC " UAA ***Stop*** UAG ***Stop***	UGU Cys (C) UGC " UGA ***Stop*** UGG Trp (W)
C	CUU Leu (L) CUC " CUA " CUG "	CCU Pro (P) CCC " CCA " CCG "	CAU His (H) CAC " CAA Gln (Q) CAG "	CGU Arg (R) CGC " CGA " CGG "
A	AUU Ile (I) AUC " AUA " **AUG** Met (M)	ACU Thr (T) ACC " ACA " ACG "	AAU Asn (N) AAC " AAA Lys (K) AAG "	AGU Ser (S) AGC " AGA Arg (R) AGG "
G	GUU Val (V) GUC " GUA " GUG "	GCU Ala (A) GCC " GCA " GCG "	GAU Asp (D) GAC " GAA Glu (E) GAG "	GGU Gly (G) GGC " GGA " GGG "

7.013 Introductory Biology
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