Name: _____

7.013 EXAM 2

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**.

NOTE: You can detach Page 6 that has the signaling pathway and a codon chart.

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

Questions	Points	Score
1	24	
2	15	
3	14	
4	14	
5	16	
6	17	
TOTAL	100	

Initials: _____

Question 1 (24 points)

Inflammation is a common manifestation of many infections and is associated with the **synthesis and secretion** of small peptides called cytokines. **Page 6** has a drawing of a signaling pathway that is triggered by the binding of cytokine ligand to the cytokine receptor (CR). ***You can detach Page 6.***

a) Circle ALL the correct option(s) for each of the following.

i. Which protein functions as a transcription factor: **Cytokine/ CR/ JAK/ STAT/ SOCS**?

ii. Location(s) of cytokine synthesis: **cytoplasm/ mitochondria/ ER membrane/ lysosomes**?

iii. The highest order of protein structure for **ACTIVE STAT**: **Primary/ secondary/ tertiary/ quaternary**?

iv. Proteins that act as kinases: **Cytokine/ CR/ JAK/ STAT/ SOCS?**

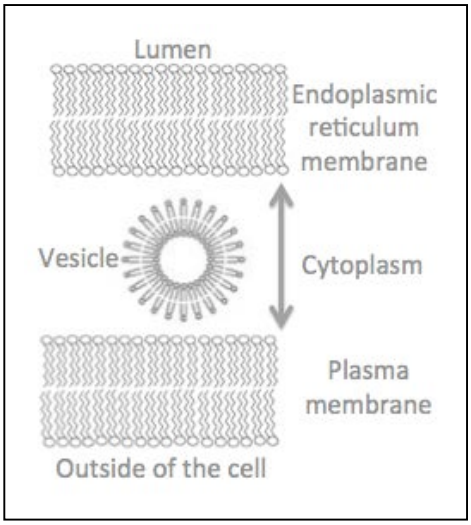
b) Consider the following **homozygous mutations** in different components of the signaling pathway.

- #1: CR lacks its extracellular domain that binds to cytokine ligand
- #2: STAT protein lacks its nuclear localization sequence
- #3: JAK protein is constitutively (always) **phosphorylated**
- #4: SOCS promoter sequence is heavily methylated

Complete the table for each of the following **mutations** in the **presence** of **cytokines**.

Mutations	**CR active (*Yes/No*?)**	**JAK protein active (*Yes/No*?)**	**STAT protein dimerized (*Yes/No*?)**	**SOCS expressed (Yes/ No)?**	**Cell Division (Yes/ No)?**
1					
2					
Both 3 & 4					

c) Assuming that CR has **ONE transmembrane domain**, on the schematic below, draw its orientation in the ER membrane, vesicle and plasma membrane and label the N and C ends of the CR on each drawing.



d) You identify a mutant cell, which produces a misfolded SOCS.

i. Propose a cellular mechanism that can refold the SOCS protein into its functional 3D- conformation.

ii. Briefly **explain** why the misfolded proteins tend to aggregate within the cytoplasm of a cell.

Initials: _____

Question 2 (15 points)

The following is the DNA sequence for the transcription initiation region of the **CR gene**. ***Note:*** *Part of the* ***promoter region*** *is boxed and the direction of transcription is shown as an →. Transcription* ***begins at*** *and includes the underlined* ***T/A*** *base pair.*

#**1** → **#2** **#3**

5'---CGGA**C**TGCTAAAATAGC**<u>T</u>**AATCGATGTG**C**TGCACG**C**AAGGCCATACGCTTGTGGTTCTT----3' **TOP**
3'---GCCT**G**ACGATTTTATCG**<u>A</u>**TTAGCTACAC**G**ACGTGC**G**TTCCGGTATGCGAACACCAAGAA----5' **BOTTOM**

a) Identify the template strand for transcription: **Top / Bottom**?

b) Write the **<u>first 6 nucleotides</u>** of the newly transcribed CR mRNA: **5'-__________________-3'**

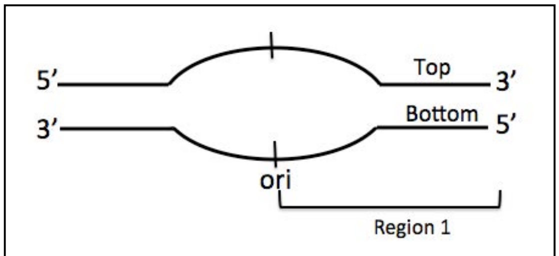
c) Write the **<u>first 2 amino acids</u>** of the newly synthesized CR protein: **N-__________________-C**

d) You create mutants 1 - 3 by substituting the C/ G base pairs (bold and shaded) at positions 1- 3 by a T/A base pair as shown on the right. Which mutant(s) (*1/ 2/ 3*) would produce a truncated CR protein? **Explain** why you selected this option and **NOT** the others.

<u>C</u> → **<u>T</u> (Top strand)**
G → **A (Bottom strand)**

Question 3 (14 points)

Below is a segment of replicating DNA from an ori site in the CR expressing skin cells.



a) On the drawing, show the direction of movement of **each replication fork** by drawing an arrow (→).

b) In **<u>Region 1</u>**, draw the primer for the leading (continuous) strand as a dashed arrow (**=>**) and label its 5' and 3' ends.

b) The following is a segment of **<u>a newly replicated DNA segment</u>**. Which base was wrongly inserted during replication: The bold and shaded **"G" <u>or</u> "A"**? **Explain** why you selected this base. ***Note:*** *The methylated Cytosine bases are bold and underlined.*

5'ATCGGGTACCGATTCT**G**AAG3' TOP
3'TAG**<u>C</u>**CCATGG**<u>C</u>**TAAGA**A**TTC5' BOTTOM

c) You grow the skin cells on plates that contain the following compounds.

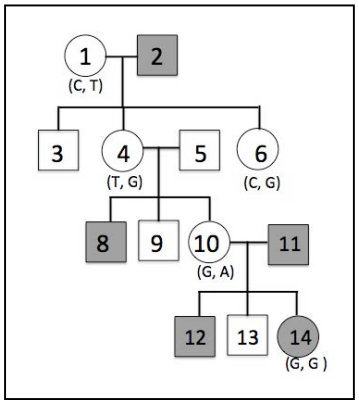
- **<u>Plate 1</u>** includes arylstibonic acid, a compound that promotes DNA supercoiling.
- **<u>Plate 2</u>** includes a drug that blocks the 3'->5' exonuclease activity of DNA polymerase.
- **<u>Plate 3</u>** includes a protease that degrades the single stranded DNA binding protein.

Which plate(s) will have cells that are **<u>NOT</u>** dividing and **why**: **1/ 2/ 3**?

Initials: _____

Question 4 (14 points)

The pedigree below shows the inheritance of hyper-inflammation due to the mutations in the CR gene. ***Note:*** #5 does not have a disease related allele. *Affected individuals are shaded. The CR-associated SNPs for some individuals are indicated.*



a) Give the **mode of inheritance** of this disease and identify the SNP(s) associated with the disease phenotype.

i. **Mode of inheritance:** _____

ii. **Disease associated SNP(s):** _____

b) You observe that the mature CR mRNA in affected individuals is longer (in bases) than that in normal healthy individuals due to disease-associated SNP. Circle **TWO** possible locations of SNP: **Promoter/ Exons/ Splice donor site/ Splice acceptor site/ 5'UTR/ 3'UTR**?

c) You create a model of the disease by using the CRISPR-Cas9 endonuclease complex. The double stranded nicks of the target sequence by CRISPR-Cas9 are an example of **hydrolysis/ condensation** reaction, which breaks the **covalent/ ionic/ hydrogen** bond.

Question 5 (16 points)

Your fellow classmate is studying families where the affected individuals do not express the CR gene. Further analysis reveals that the enhancer sequence corresponding to CR gene in these patients cannot bind to specific transcription factors. She wants to further characterize the enhancer sequence.

a) Which library should she use to identify the bacterial clone carrying the enhancer sequence specific to CR gene in affected and healthy individuals: The **genomic/ skin cell cDNA/ skin cell expression** library? **Why?**

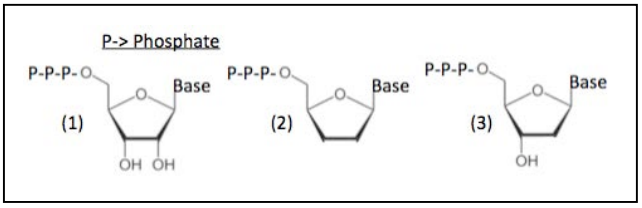
b) She isolates the plasmid that has the enhancer sequence insert and PCR amplifies the enhancer sequence (shown below) from affected and healthy individuals. Give the sequence of the 6-bases long primer to make the….

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

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

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

c) She sequences the PCR amplified enhancer sequence. Which of the following nucleotides is used in DNA sequencing but NOT in PCR and **why**?



d) She finds that the sequence of the CR enhancer in affected and healthy individuals is the SAME. Select an alternative mechanism from below that explains why CR gene is **not transcribed** in patients.

i. Mature mRNA corresponding to CR gene lacks the 7- Methyl-Guanine at its 5' end

ii. DNA demethylase removes the methyl group from the bases in the enhancer sequence

iii. Histone proteins bound to the CR- enhancer region are de-acetylated

Question 6 (17 points)
You would like to understand where the CR protein is localized in the cell, and whether its location changes with inflammation. To do this you plan to ligate the cDNA sequence corresponding to the C-terminus of CR gene with the cDNA sequence corresponding to the N- terminus of GFP gene to make a **CR-GFP fusion cDNA** that encodes the **CR-GFP fusion protein**.

.

a) What part of the **CR-GFP fusion protein** can inform you of its location in the cell? ________

The following is the partial cDNA sequence encoding the C- terminus of the CR gene. ***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
CR:    5'AAA ATT CTG CAG AAT ACA ATT CCG CTG CAG TAG TTT GAA TTC ATC3'
       3'TTT TAA GAC GTC TTA TGT TAA GGC GAC GTC ATC AAA CTT AAG TAG5'
```

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'
```

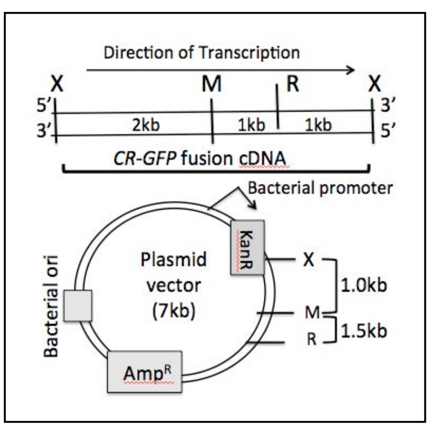
The recognition sequences and the cleavage sites (*indicated by /*) for each enzyme are given below.

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'

b) Complete the table below for each pair of restriction enzyme.

Restriction enzyme pair used to digest CR and GFP cDNAs	Can you clone and express the CR-GFP fusion cDNA in the bacteria? **Why or why not?**
1 & 2	
3 & 4	
5 & 5	

You clone the CR-GFP fusion gene into the following plasmid and use it to transform the bacteria. ***Note:*** *Both the CR-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.*

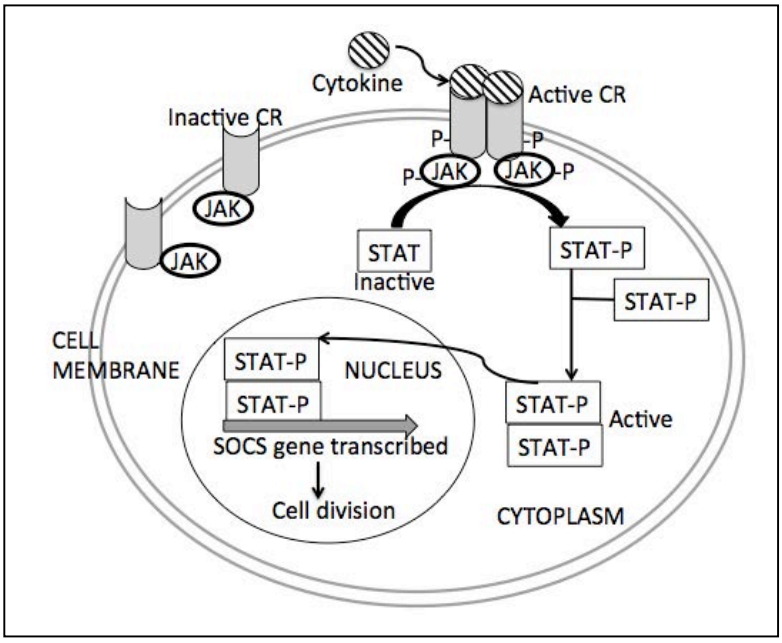


c) How would you **select and screen** for bacterial colonies that have the recombinant plasmid?

d) You analyze two bacterial colonies that have the recombinant plasmid with the CR-GFP insert. Which restriction enzyme would you use to determine the orientation of the CR-GFP insert within the recombinant plasmid**: X/ M/ R**? **Explain,** why you selected this option.

Signaling pathway for Question 1

Step 1: Cytokine receptors (CR) remain bound to JAK proteins and they are both dephosphorylated when inactive.



Step 2: Binding of cytokines to CR causes the dimerization of CR.

Step 3: JAK proteins bound to the cytoplasmic domains of the CR phosphorylate each other (shown as –P). They also phosphorylate and activate the dimerized CR.

Step 4: Activated CR phosphorylates and dimerizes STAT proteins and this results in its activation.

Step 5: Active **STAT dimer** moves to the nucleus and promotes the transcription of the *SOCS* gene by binding to the *SOCS* promoter sequence. This promotes cell proliferation.

Codon Chart

	U	C	A	G	
U	UUU **phe**	UCU **ser**	UAU **tyr**	UGU **cys**	U
	UUC **phe**	UCC **ser**	UAC **tyr**	UGC **cys**	C
	UUA **leu**	UCA **ser**	**UAA STOP**	**UGA STOP**	A
	UUG **leu**	UCG **ser**	**UAG STOP**	UGG **trp**	G
C	CUU **leu**	CCU **pro**	CAU **his**	CGU **arg**	U
	CUC **leu**	CCC **pro**	CAC **his**	CGC **arg**	C
	CUA **leu**	CCA **pro**	CAA **gln**	CGA **arg**	A
	CUG **leu**	CCG **pro**	CAG **gln**	CGG **arg**	G
A	AUU **ile**	ACU **thr**	AAU **asn**	AGU **ser**	U
	AUC **ile**	ACC **thr**	AAC **asn**	AGC **ser**	C
	AUA **ile**	ACA **thr**	AAA **lys**	AGA **arg**	A
	AUG met	ACG **thr**	AAG **lys**	AGG **arg**	G
G	GUU **val**	GCU **ala**	GAU **asp**	GGU **gly**	U
	GUC **val**	GCC **ala**	GAC **asp**	GGC **gly**	C
	GUA **val**	GCA **ala**	GAA **glu**	GGA **gly**	A
	GUG **val**	GCG **ala**	GAG **glu**	GGG **gly**	G

7.013 Introductory Biology

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