



1. (10 points) From the following list circle all of the components required for **translation** in *E. coli*. (Note that points will be subtracted for incorrect answers).

RNA polymerase    ribosomes    mRNA    tRNA    ATP    GTP  
dATP,dTTP,dCTP,dGTP    DNA polymerase I    amino acids    ligase  
reverse transcriptase    aminoacyl-tRNA synthase    DNA polymerase III    ribosomes  
initiation factors    RNA-dependent polymerase    peptidoglycan    glucose  
elongation factors    sigma factor    termination factors    primase    helicase  
single-stranded DNA binding protein    DNA

From the following list circle all of the components required for **replication of DNA** in *E. coli*. (Note that points will be subtracted for incorrect answers).

RNA polymerase    ribosomes    mRNA    tRNA    ATP    GTP  
dATP,dTTP,dCTP,dGTP    DNA polymerase I    amino acids    ligase  
reverse transcriptase    aminoacyl-tRNA synthase    DNA polymerase III  
initiation factors    RNA-dependent polymerase    peptidoglycan    glucose  
elongation factors    sigma factor    termination factors    primase    helicase  
single-stranded DNA binding protein    DNA

2. (6 points) In answering the questions below list as many proteins as are necessary; if none are required state Anone@.

What proteins does a bacterium require for the transcription of vegetative genes?

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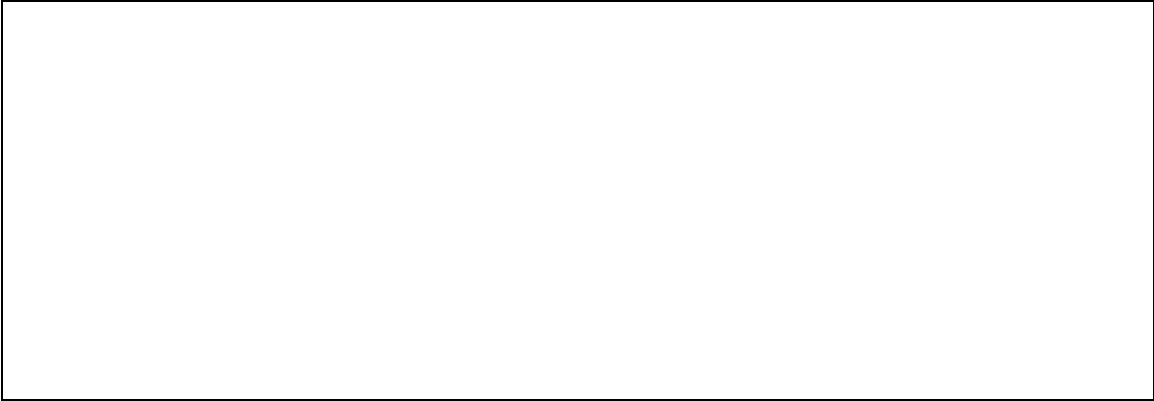
What virus-encoded proteins does polio require for the transcription of its genes?

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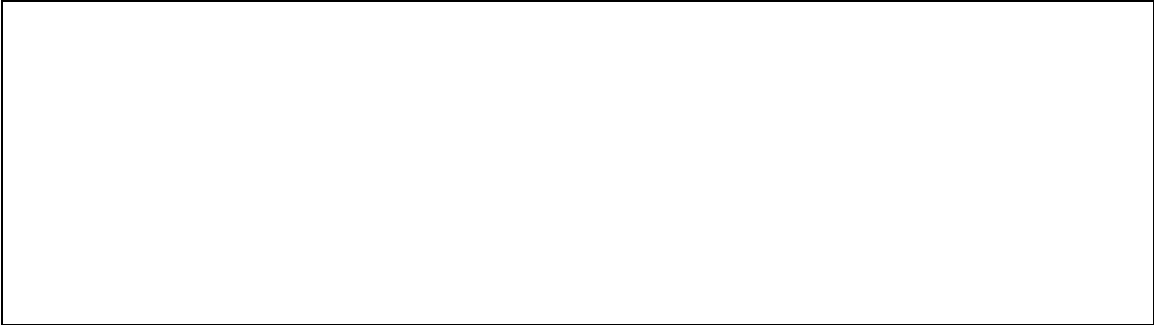
What virus encoded proteins does T7 need for transcription of **late** genes?

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3. (14 points) RNA viruses that grow in plant or animal cells have to solve the problem that eukaryotic cells generally only make one protein using one piece of mRNA. Different viruses have used different strategies to circumvent this problem. Describe the strategy used by tobacco mosaic virus (a tobamo virus).



Describe the strategy used by influenza virus.



Use the following information for the remainder of the questions on the exam.

**amino acids**

threonine (thr)  
leucine (leu)  
histidine (his)  
tryptophan (trp)  
arginine (arg)

**vitamins**

biotin (bio)  
thiamine (thi)

**sugars**

glucose (glu)  
lactose (lac)  
maltose (mal)  
arabinose (ara)

**antibiotics**

streptomycin (sm)  
rifampicin (rif)  
ampicillin (amp)  
tetracycline (tet)

4. ( 9 points) You perform an conjugation between two *E. coli* strains with the following genotypes: F:  $sm^R, thr^-, his^-, thi^-, mal^-, lac^+$

Hfr :  $sm^S, thr^+, his^+, thi^+, mal^+, lac^+$

You have available the ingredients listed above as well as minimal medium and complex medium. What medium would you use to select transconjugants of each of the following genotypes?

$thr^+$  \_\_\_\_\_

$mal^+$  \_\_\_\_\_

$thi^+$  \_\_\_\_\_

5. (9 points) You perform an interrupted mating between two *E. coli* strains with the following genotypes: F:  $rif^R arg^-, leu^-, thi^-, ara^-$   
 Hfr:  $rif^S arg^+, leu^+, thi^+, ara^+$

The conjugates are plated on the following minimal media at the times indicated:

Media 1: leu, thi, glu, rif

Media 2: arg, leu, glu, rif

Media 3: arg, thi, leu, ara, rif

Time of interrupted Mating	# of colonies		
	Media 1	Media 2	Media 3
5 min	0	0	0
10 min	0	0	4
15 min	2	9	24
20 min	12	26	44
30 min	32	56	84

Graph these data on the supplied graph paper. Be sure to label the axes and to indicate which line represents which data.

Given the above data label the order of gene transfer below (1<sup>st</sup>, 2<sup>nd</sup>, or 3<sup>rd</sup>):

*arg* \_\_\_\_\_  
*thi* \_\_\_\_\_  
*ara* \_\_\_\_\_

What is the time of transfer of each gene (in min.)?

*arg* \_\_\_\_\_  
*thi* \_\_\_\_\_  
*ara* \_\_\_\_\_

6. ( 10 points) You wish to determine the gene order and relative distances of three genes from *E. coli*. You prepare DNA from *E. coli* which is *his<sup>-</sup> thi<sup>+</sup> mal<sup>+</sup>* and use it to transform *E. coli* which is *his<sup>+</sup> thi<sup>-</sup> mal<sup>-</sup>*. You select for cells which are *thi<sup>+</sup>* or *mal<sup>+</sup>* and test them for the ability to make a his or thi, or to grow on maltose. You obtain the following results:

Selected marker	Percentage of selected cells which are		
	<i>his<sup>+</sup></i>	<i>thi<sup>+</sup></i>	<i>mal<sup>+</sup></i>
<i>thi<sup>+</sup></i>	80	100	30
<i>mal<sup>+</sup></i>	30	30	100

Draw a diagram to show the gene order and relative positions

What medium did you use to test whether the *thi<sup>+</sup>* bacteria are *his<sup>+</sup>*?

Why didn't you place the original transformation on media to select for *his<sup>+</sup>* cells

For the next two questions you have all of the following available:

competent *E. coli lac*<sup>-</sup>

your bacterium

competent your bacterium

DNA of plasmid pUGH, which contains an origin of replication recognized by *E. coli* but not by your bacterium and a transposon Tn5 which encodes *sm*<sup>R</sup>

plasmid p108

restriction enzymes and all other necessary enzymes and small molecules and buffers

X-gal

the toxic chemical

minimal medium

complex medium

all of the chemicals, etc. listed on page

wheat plants and medium in which to grow them

\_ phage

mice

7. (9 points) You find a bacterium which can grow on a toxic chemical (using it as a carbon source) in the soil at a superfund site. You are interested to identify the genes required for growth on this chemical and decide to use transposon mutagenesis to identify these genes. Given the following things, fill in the blanks in a protocol for transposon mutagenesis that will enable you to find these genes.

Fill in the blanks in the protocol below for isolating transposon mutants of your bacterium which are unable to grow on the toxic chemical.

1. Introduce \_\_\_\_\_ into \_\_\_\_\_ by \_\_\_\_\_ (process)

2. Grow the result on \_\_\_\_\_ medium containing \_\_\_\_\_.

3. To identify mutants which can no longer grow on the toxic chemical do the following:

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4. In step 2, you are selecting/screening (circle one) for bacteria which carry the transposon

5. In step 3, you selecting/screening (circle one) for the particular mutants you wish to obtain.

8. (14 points) Your lab partner has the same materials available in her lab. She decides to use gene cloning instead of transposon mutagenesis to try to find the genes required for growth on the toxic chemical.

Fill in the missing steps in her protocol.

1. Isolate \_\_\_\_\_ from \_\_\_\_\_ and digest it with \_\_\_\_\_.

Do a partial or complete (circle one) digest.

2. Isolate \_\_\_\_\_ from \_\_\_\_\_ and digest it with \_\_\_\_\_.

Do a partial or complete (circle one) digest.

3. Mix the products of #s 1 and 2 and add \_\_\_\_\_(enzyme).

4. \_\_\_\_\_ the product of # 3 into \_\_\_\_\_(cells).

5. Plate the resulting cells on \_\_\_\_\_ medium containing

\_\_\_\_\_.

Keep the \_\_\_\_\_ colonies.

These colonies contain the genes required for growth on the toxic chemical.

9. (11 points) You are doing a survey of various strains of a bacterium which can produce a toxin. You wish to determine which isolated carry the toxin gene.

For this purpose you decide to use pcr. You have sequenced the toxin gene from strain 1. The partial sequence is shown below:

5'ATGCCGTGGCAGGACTTCGC.....GTGCAGCGATTGCGCATTGCC 3'  
3' TACGGCACCGTCCTGAAGCG.....CACGTCGCTAACGCGTAACGG 5'

The dots represent the middle of the gene.

Given the following materials fill the protocol shown for obtaining the DNA encoding the gene by pcr from your various strains.

oligonucleotide 1 5'ATGCCGTGGCAGGACTTCGC 3'

oligonucleotide 2 5' GTGCAGCGATTGCGCATTGCC 3'

oligonucleotide 3 3' TACGGCACCGTCCTGAAGCG 5'

oligonucleotide 4 3' CACGTCGCTAACGCGTAACGG 5'

your strains of bacteria

ligase

competent *E. coli*

ATP

GTP

*E. coli* DNA polymerase

*Thermobacterium aquaticus* DNA polymerase

dATP,dCTP, dGTP, dTTP

ATP, GTP, CTP, UTP

buffers, etc.

a heating block with a programable temperature control

1. Extract and purify \_\_\_\_\_ from \_\_\_\_\_.

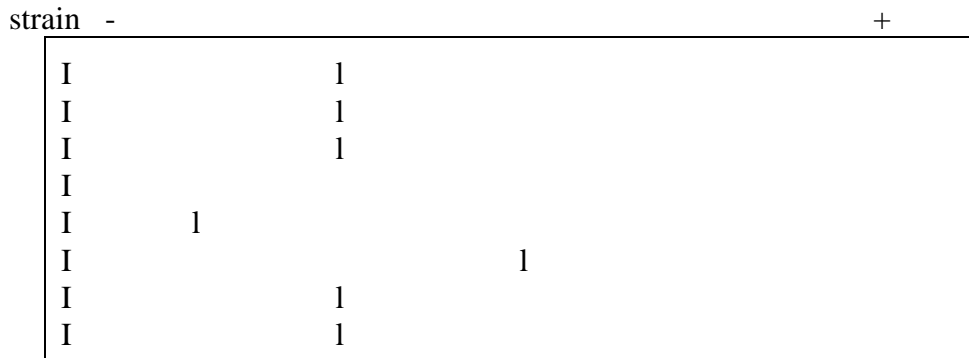
2. Add to the purified material the following :\_\_\_\_\_.

3. Program the heating block to repeat the following cycle about 30 times:

\_\_\_\_\_ and

incubate your reactions in the heat block.

You now have your PCR products and decide to analyze them using gel electrophoresis. You obtain the following results:



Where the I represent the well where the sample was applied to the gel and 1 represents the stained DNA band observed. Remember that strain 1 was the strain from which the DNA sequence was obtained.

What can you say about the toxin gene in strains 2, 3, 7, and 8? \_\_\_\_\_

What can you say about the toxin gene in strain 4? \_\_\_\_\_

What can you say about the toxin gene in strain 5? \_\_\_\_\_

What can you say about the toxin gene in strain 6? \_\_\_\_\_