1. An experiment was designed to obtain nonspecific transcription from both strands of a DNA molecule. Which of the following strategies would be most effective in achieving this?
   A. Include the RNA holoenzyme in the reaction.
   B. Use the core enzyme of RNA polymerase.
   C. Enrich the preparation with sigma subunit.
   D. Choices A and B are both effective.

2. Which of the following is not present in the core RNA polymerase?
   A. $\beta$
   B. $\beta'$
   C. $\sigma$
   D. both $\beta$ and $\sigma$

3. Place the following in the order that they occur during transcription initiation:
   1 - formation of open complex; 2 - formation of closed complex;
   3 promoter clearance; 4 - synthesis of about 10 nucleotides
   A. 1, 2, 3, 4  B. 2, 1, 4, 3  C. 2, 1, 3, 4  D. 3, 1, 2, 4  E. 3, 2, 1, 4

4. Which of the following is most involved in elongation of the RNA transcript?
   A. core polymerase
   B. sigma factor
   C. -10 region
   D. both sigma factor and -10 region

5. Which of the following is/are characteristic of intrinsic terminator elements?
   A. They contain an inverted repeat.
   B. They contain a hairpin loop.
   C. They contain several T's in the nontemplate strand of DNA.
   D. Only choices A and B are correct.
   E. Choices A, B and C are correct.

6. Which of the following statements is true about a lac operon with the following genotype?
   \[ I \ O^c \ Z^+ \ Y^+ / I \ O^+ \ Z \ Y \]
   A. A repressor protein will be constitutively produced.
   B. $\beta$-galactosidase will be constitutively produced.
   C. A cis-dominant mutation is present.
   D. Permease will be constitutively produced.
   E. Choices B and C are correct.

7. Which of the following plasmids could be used to restore inducible regulation of $\beta$-galatosidase in this mutant: $I^c \ O^c \ Z \ Y^+ \ A^+$?
   A. $I^+ \ O^c \ Z \ Y^+ \ A^+$
   B. $I^+ \ O^c \ Z \ Y \ A^+$
   C. $I \ O^+ \ Z^+ \ Y^+ \ A^+$
   D. $I \ O^c \ Z^+ \ Y^+ \ A^+$

Multiple choice have one choice each and are worth 2.5 pts each.
8. Which of the following is true about the action of CAP at the lac promoter?
A. CAP monomer binds directly to the promoter stimulating polymerase to bind.
B. CAP-cAMP blocks recruitment of polymerase to the promoter.
C. CAP blocks the αCTD of RNA polymerase.
D. Binding of the CAP-cAMP to the lac activator-binding site recruits RNA polymerase.
E. Choices C and D are correct.

9. Which of the following is an example of an allosteric interaction?
A. allolactose binding to lac repressor
B. tryptophan binding to trp repressor
C. polymerase binding to the promoter
D. repressor binding to the operator
E. Choices A and B are correct.

10. Which of the following explains the events in late stage of phage SP01 infection in bacteria?
A. The host sigma factor specifies transcription of genes.
B. There is a high level of transcription of host genes.
C. Host polymerase holoenzyme directs transcription.
D. Host core enzyme participates in transcription in conjunction with phage-encoded specificity factors.
E. All of the choices are correct.

11. Which of the following is most likely to occur when sporulation occurs in B. subtilis?
A. complete shutdown of transcription
B. activation of vegetative genes
C. deletion of vegetative genes
D. complex sigma-switching
E. reduction of endospore formation

12. An experiment is planned to look at the in vivo transcription start site for the spoIID gene during sporulation. Which of the following techniques would yield the most information in this experiment?
A. Southern analysis
B. Northern analysis
C. S1 mapping
D. DNAse footprinting
E. Choices B and C will be the most useful

13. E. coli RNA polymerase differs from DNA polymerase in that it:
A. synthesizes new strands of RNA in a 3’ to 5’ direction
B. is a monomeric protein.
C. can synthesize a complementary strand without the two strands of DNA being separated.
D. does not require a primer to initiate synthesis or RNA
14. The regions of the DNA where RNA polymerase binds can be identified by:
   A. restriction mapping  B. Southern blot  C. DNase footprinting  D. PCR

15. Predict the outcome of infection by λ phage mutant in which the N gene was deleted:
   A. lysogenic cycle will be induced.
   B. entry into the bacterial host will be blocked.
   C. antitermination will be reduced.
   D. transcription from the left promoter will be interrupted.
   E. Both choices C and D are correct.

16. Which of the following is true about the early phase of T7 infection of bacteria?
   A. Class II genes are the first to be transcribed.
   B. The host polymerase transcribes class II genes.
   C. The phage-encoded polymerase transcribes the class I phage genes.
   D. Class I genes are the first to be transcribed.

17. During an experiment to study infection of bacteria with lambda, the bacterial cultures were accidentally exposed to a UV light source. What would be expected after this exposure?
   A. recA gene is turned off.
   B. Coprotease activity in RecA protein is activated.
   C. SOS response is induced.
   D. Choices B and C are correct.
   E. Choices A and C are correct.

18. E. coli lysogenized with lambda cannot be superinfected by another lambda because:
   A. the superinfecting phage DNA is rapidly degraded by the host
   B. the superinfecting phage DNA is inhibited by excess repressor binding
   C. the superinfecting phage cannot replicate due to insufficient nucleotide precursors
   D. Choices A and C are correct

19. (10 pts) Enzymes Matching. One choice each; choices are used only once.
   ____ T7 polymerase  a. causes termination by RNAP by binding transcripts
   ____ topoisomerase  b. transports lactose into cell
   ____ lac permease  c. single subunit RNA polymerase
   ____ B-galactosidase  d. changes superhelical form of DNA
   ____ Rho  e. cleaves DNA at specific sites
             f. cleaves lactose to glucose plus galactose

20. (10 pts) Matching. Weak promoters can be activated in different ways. One choice each.
   ____ λ repressor and P_RM  a. bound protein binds B, B' of RNAP
   ____ CAP protein + cAMP  b. bound protein interacts with alpha subunit of RNAP
   ____ λ PRE promoter  c. cII protein binds to promoter and stimulates RNAP
   ____ AraC protein  d. bound protein interacts with sigma factor of holoenzyme
   ____ T7 promoter  e. inducer reduces affinity for upstream operator; breaks repression loop.
             f. new single subunit polymerase with specificity for the promoter
21. (3 pts) You infect two genetically different strains of *E. coli* with wild-type lambda phage, and select lysogens in each strain. Southern blot verifies that both strains contain lambda DNA inserted at the typical position. When you irradiate these lysogens with UV light, you get lytic infection from one strain (strain A), but nothing from the other (strain B). Briefly explain these results.

22. (12 pts) Draw the structure of a typical bacterial gene: include promoter (-10 and -35 regions, transcription start), terminator, 5’-UTR, 3’-UTR, coding region. Also indicate the typical location for the binding site for a repressor protein, and the typical location for the binding site for a positive activator.

23. (6 pts) This plasmid contains two promoters:
a vegetative promoter (veg)
and a sporulation promoter (Spo).
The plasmid is cut with EcoRI,
which cuts 500-bp downstream of the veg promoter,
and 300 bp downstream of the Spo promoter.
If *in vitro* RNA synthesis reactions are performed
with purified RNAP plus the indicated σ factor(s):
diagram on the gel the expected sizes of RNA fragments
(Marker fragments are indicated)

24. (8 pts) Explain the following findings:
(1) core RNAP transcribes intact T4 phage DNA only weakly, whereas holoenzyme transcribes this template very well;
but (2) core RNAP can transcribe calf thymus DNA about as well as the holoenzyme can.
25. (8 pts) Draw diagrams of the *lac* operon that illustrate (a) negative control and (b) positive control. Briefly explain why are negative and positive control of the *lac* operon important to the energy efficiency of *E. coli* cells.

26. (10 pts) You are studying a new operon in *E. coli* involved in phenylalanine biosynthesis.
   a. How would you predict this operon is regulated (inducible or repressible by phenylalanine, positive or negative)? Why?

   b. You sequence the operon and discover it has a short open reading frame near the 5’-end of the operon that contains several codons for phenylalanine. What prediction would you make about his leader sequence and the peptide that it encodes?

   c. What would happen to the gene regulation if the sequence of this leader were changed so that the phenylalanine codons (UUU, UUU) were changed to leucine codons (UUA, UUG)?

   d. What is this kind of transcriptional regulation called? ________________
   Why would this type of regulation not work in a eukaryotic cell?
27. (8 pts) **Attenuation** (trp operon) and **antitermination** (N protein, lambda) both regulate transcription termination. Compare/contrast these processes in terms of their effects on elongation of nascent RNA transcripts and the molecular mechanisms by which they occur.

28. (6 pts) Special cloning systems. You have cloned the gene for human growth hormone (HGH) in a plasmid, and expressed the gene from a T7 promoter. You have a separate plasmid containing the cloned gene for T7 RNA polymerase, expressed from the *lac* promoter/operator region. You put both plasmids into an *E. coli* cell that contains a normal *lac* operon.

You grow the cells in Luria broth (LB), which contains nutrients, but little glucose and which does not give rise to catabolite repression (via CAP protein).

In the table, indicate what you expect for the expression of **B-galactosidase**, **T7 polymerase** and **HGH** (use relative terms like none, low, medium, high)

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<thead>
<tr>
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<th>B-galactosidase</th>
<th>T7 polymerase</th>
<th>HGH</th>
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<tr>
<td>Basic LB media</td>
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<tr>
<td>LB Media containing IPTG</td>
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29. (4 pts) What was most unusual or surprising about the topics covered in this section? Briefly explain.