1. (3) Show the fragments resulting from digestion with the restriction enzyme EcoRI. Make sure and indicate the 5’ phosphates and 3’ hydroxyls.

EcoRI has the following cutting specificity: 5’ GAATTC 3’

5’ GTCAATTTCGAAGTCAACG-OH
3’ CAGTTAAAGCTTCAGTGTTTAAGCCGTTTTAACCAGCATGCAGTA 5’

2. (2) The Cohen and Boyer experiment was the first demonstration that:
   a) Restriction enzymes could cut DNA
   b) Foreign DNA could be cloned. answer: b
   c) PCR could be used to amplify DNA fragments.
   d) DNA ligase was required to covalently link two DNA strands

3. (6) Describe the steps needed to carry out one round of PCR. Please include in your description what happens during each step and why it is needed.

PCR reaction contains: DNA template, 2 primers, buffer, nucleotides and taq polymerase

1. Heat the reaction up to ~95ºC to denature the template strands.

2. Cool the reaction down to near the T_m of the primer-template duplex so that the primers can hybridize to the template.

3. Warm the reaction up to ~95ºC so that taq polymerase can synthesize DNA from the primers.
4. (6) Match each technique with its correct use.

6. a) Run-on transcription 1. Determine whether a protein binds DNA
4. b) Primer extension 2. Determine the size and abundance of a RNA
5. c) Southern blot 3. Determine where a protein binds in a DNA fragment
2. d) Northern blot 4. Determine the start of transcription
3. e) DNase footprinting 5. Determine if a probe is complementary to a DNA target
1. f) Gel mobility shift assay 6. Determine the transcription rate

5. (4) RNA polymerase ___II_____ is the most sensitive α-amanatin and is used to transcribe
_____mRNA_____(class of RNA), whereas RNA polymerase ___I____ is the least
 to α-amanatin and is used to transcribe _____rRNA_______(class of RNA).

6. (4) Describe an experiment that shows the TATA box determines the transcription start site
for a typical Pol II promoter.

Start with a wild type TATA box containing promoter and determine the transcription start site
via primer extension. Make mutant promoters that either remove the natural transcription start
site or delete sequences between the natural transcription start site and the TATA box, then
measure the transcription start site from these mutants via primer extension. Mutants will
continue to initiate transcription approximately 25bp from the TATA box.

7. (4) Draw a typical Pol I promoter. Be sure to indicate the spacing of regulatory elements and
transcription start site.

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UCE
-156

Core
-107
-45
+20
+1


8. (3) Several modified genes have been made and tested to determine their levels of transcription: highly transcribed (+ ++); moderate transcription (+ +); and basal transcription (+). Deleted portions of the gene are indicated by dashed lines and exons are indicated as boxes. Indicate the positions of enhancers and silencers involved in regulating this gene.

- [Diagram showing transcription levels and enhancers/silencers]

9. (2) TFIIS functions to: answer: d
   a) Bind Sp1
   b) Phosphorylate TFIIH
   c) Unwind DNA
d) Stimulate RNA proofreading
e) Terminates transcription

10. (7) Describe or draw the order of binding for each of the factors required to form a Pol II preinitiation complex.

1) TFIID binds weakly to the TATA box
2) TFIIA binds to TFIID and DNA to enhance TFIID binding
3) TFIIB binds to the TFIID/TFIIA complex
4) PolII is always associated with TFIIF
5) Pol II/TFIIF binds to the DAB complex.
6) TFIIE binds to the DABPol II/F complex
7) TFIIH binds to the complex, thus forming a mature preinitiation complex.

11. (4) TFIIH is comprised of a ____4____ subunit kinase domain that phosphorylates the carboxy terminal tail of ____RNA Pol II____, and a five subunit ____helicase____ domain that unwinds DNA strands required for ____promoter____ clearance.
12. (6) For each RNA polymerase, TAF containing complexes are situated just upstream of the transcription start site. Name these complexes for:

Pol I  - SL1
Pol II - TFIID
Pol III - TFIIB

13. (6) List three differences between tRNA and 5S rRNA transcription initiation.

1) TFIIIA is required for 5S rRNA transcription, but not tRNA transcription.
2) An “A” box is required for 5S rRNA transcription, but not tRNA transcription.
3) A “B” box is required for tRNA transcription, but not 5S rRNA transcription.

14. (7) Match the terms on the left with the correct descriptor on the right. There is one correct descriptor for each term.

4   a) Zinc fingers  1. comprised of a helix-turn-helix and a recognition helix
3   b) ZIP domain   2. releases hsp90 upon hormone binding
1   c) Homeodomain  3. coiled coil formed by interactions among leucines
5   d) Basic domain  4. requires two cysteines and two histidines to form
6   e) HLH domain   5. comprised of many positively charged amino acids
7   f) Activation domain  6. mediates dimerization via two helices interrupted by a loop
2   g) Nuclear receptor 7. comprised of many negatively charged amino acids.

15. (2) What sort of factor blocks enhancers and silencers from acting on inappropriate genes?

An insulator serves to block enhancers and silencers from acting on inappropriate genes.

16. (3) Describe in general terms how a transcriptional activator promotes transcription initiation once it binds to an enhancer element.

Once a transcriptional activator is bound to the enhancer, its activation domain then interacts with some component of the preinitiation complex (i.e. TFIID, TFIIB, etc.) to promote the formation of a preinitiation complex on the promoter so that transcription can begin.
17. (6) Describe an experiment showing that enhancers do not have to be on the same piece of DNA to enhance transcription.

Four different plasmids are generated for this experiment (1) a positive control with the enhancer and promoter on the same plasmid. (2) a negative control that consists of a plasmid with a promoter and a separate plasmid with the enhancer, and (3) an experimental plasmid with a promoter, an enhancer, and two recombination sites between the promoter and the enhancer.

Recombination of the experimental plasmid leads to the formation of a catenane, in which the two resulting plasmids are linked to each other. When the experimental and control plasmids are transfected into cells, transcription from the promoter is measured with the following results.

The negative control shows no enhancement of transcription of the promoter on one plasmid from an enhancer on a completely separate plasmid.

The positive control plasmid shows strong enhancement of transcription from the enhancer on the same plasmid.

The experimental shows enhancement of transcription from the promoter due to the enhancer on the separate (but linked) plasmid containing the enhancer.

18. (2) What is the difference between RNA polymerase II and RNA polymerase II holoenzyme?

RNA Pol II consists of the proteins that form the polymerase enzyme itself, whereas the RNA Pol II holoenzyme contains not only the polymerase enzyme, but also many, if not all components of the preinitiation complex (i.e. TFIID, TFIIA, TFIIB, etc.).

19. (5) Describe how the mediator CBP promotes transcriptional activation by a nuclear receptor.

1) a nuclear receptor must first be bound by its hormone ligand to promote nuclear localization and DNA binding
2) binding hormone also alters the conformation of the nuclear receptor so that it forms a CBP binding site
3) CBP binds to the nuclear receptor
4) CBP then interacts with a component of the preinitiation complex
5) The preinitiation complex then binds to the promoter and transcription can begin
20. (6) A nucleosome is comprised of two copies of histones __H2A__, __H2B__, __H3__, __H4__, one copy of histone __H1__, and __200__ bp of DNA.

21. (4) The second order of chromatin folding is __30nm__ fibers, which form at __high/increased__ ionic strength due to interactions between __H1__ and __H1__.

22. (4) In the third order of chromatin folding is due to the formation of 35kb to 85kb __loops__ that are attached to __Topoisomerase/chromosome scaffold__ at their base and are supercoiled.

23. (2) The repressive affects of histone H1 on transcription can be counteracted by __transcriptional activators__.

24. (2) What is the function of an antirepressor?

An antirepressor either moves nucleosomes that obscure a promoter or it keeps them from obscuring the promoter.