(96)

MCB 102 Name _____

~~(6 pages total) (100 points total~~

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OLD **FINAL EXAMINATIONS**

I. The restriction enzyme *AvaII* is purified from *Anabaena variabilis* and is available commercially. It recognizes the following DNA sequence:

$$\begin{array}{llllll} 5' & G\downarrow G & \begin{matrix}A\\T\end{matrix} & C & C & 3' \\ 3' & C & C & \begin{matrix}T\\A\end{matrix} & G\uparrow G & 5' \end{array}$$

a.) (5 points) How many fragments would you expect to observe if this enzyme were used to cut phage DNA. Assume the phage DNA to be 50 kb in size, double stranded, linear, unmethylated, and to contain about 50% (G + C).

Probability for each base =

$(.25) \times (.25) \times (.5) \times (.25) \times (.25) = 0.001953 \times 50{,}000$ bases (50 kb)

$= 97.6$

$\cong 98$

b.) (5 points) On further examination, it was observed that the phage DNA only contained 30% (G + C). What would be your revised prediction of the number of fragments observed?

Probability for each base =

$(.15) \times (.15) \times (.7) \times (.15) \times (.15) = 0.00035543 \times 50{,}000$ bases

$= 17.7$

$\cong 18$

II. Explain briefly why:

1. (5 points) DNA polymerase III holoenzyme activity was so difficult to detect in bacterial extracts for early workers on DNA replication.

Most of the DNA polymerizing activity in *E. coli* is due to DNA polymerase I. DNA polymerase III activity is very low by comparison (there are only about 10 molecules/cell). Until *polA* mutants were isolated, it wasn't possible to detect this activity.

II. Explain briefly why (continued)

2. (5 points) Ethidium bromide, when added at low levels to negatively supercoiled plasmid DNA (B-form), can relax the supercoils. However, when the same concentration of ethidium bromide is added to positively supercoiled plasmid DNA (B-form), it will increase the number of supercoils.

Ethidium bromide interpolates between the DNA strands and unwinds the DNA slightly. This changes the number of bases per turn of the helix. Negatively supercoiled plasmid DNA is strained because it is underwound. Adding ethidium ~~by~~ reduces this strain since it reduces the ~~no~~ amount of the "underwinding." Positively supercoiled DNA is "overwound." Increasing the number of bases per turn of the helix will only make this problem worse - it further increases the strain on the DNA.

3. (5 points) The 5'→3' exonuclease activity of DNA polymerase I is necessary for DNA replication in *E. coli*.

This activity is needed for nick translation. The activity removes RNA primers in the DNA and ~~repairs~~ removes DNA with errors.

4. (5 points) DNA sequencing using the Sanger technique and dATP-[^{32}P] as a labeled precursor requires that the reactions are carried out in four separate tubes.

The principle of the Sanger method is that random termination occurs when a template is replicated and a respective dideoxy nucleotide is incorporated. The size of the fragment and the knowledge of the dideoxy base incorporated lets you know which base is present at a particular position. If all the dideoxy nucleotides were added at the same time, you would not know which base was specific to any position.

5. (5 points) Transcription of the tryptophan operon of *E. coli* is turned off almost immediately following the addition of high levels of tryptophan to the culture medium.

The tryptophan repressor (plus tryptophan) represses transcription by binding ~~the~~ to the operator/promoter region of the DNA upstream of the trp operon. This binding prevents RNA polymerase from binding to the promoter.

II. Explain briefly why (continued)

6. (5 points) In higher eukaryotes, the mRNA transcript is rapidly spliced by the splisosome, but the DNA template (which has the identical sequence) is never spliced, even when the DNA is single stranded.

Splicing usually requires the formation of a lariat containing a 5'→2' phosphodiester bond. Since DNA does not contain a free 2'-OH group, this reaction is limited to RNA.

7. (5 points) Most eukaryotic mRNA is polyadenylated at its 3' end.

Polyadenylation of mRNA allows it to bind the PAB I protein. This (a) stabilizes the mRNA and protects it from nucleases. (b) it also is important for initiating protein synthesis.

8. (5 points) Periplasmic proteins of *E. coli* are significantly smaller than predicted from their DNA sequence.

Periplasmic proteins are proteins that are transported through the membrane and are secreted into the "periplasmic space". These proteins contain a signal sequence, about 20 amino acids at their N-terminus. These amino acids are removed by a signal protease (peptidase). Thus, the mature (transported) proteins are smaller than predicted.

9. (5 points) Many AUG codons in *E. coli* are never recognized by prokaryotic ribosomes as sites for the initiation of protein synthesis, others are recognized intermittently, and others still are recognized very often.

AUG codons not preceded by a Shine-Dalgarno sequence are not recognized very well by E. coli ribosomes.
AUG codons preceded by consensus Shine-Dalgarno sequence are expressed highly.
Intermittent recognition may occur if the mRNA is involved in secondary 137 structure (base-pairing). This can block ribosome binding.

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II. Explain briefly why (continued)

10. (5 points) The leader region of the histidine operon codes for a peptide rich in histidine (7 histidine codons).

The leader region contains sequences which can form secondary mRNA structures, one of which causes termination (stem structure followed by UUUUU). It also encodes a peptide rich in histidine. Cells starved for histidine are low in $tRNA^{his}$. The ribosomes stall when translating the leader peptide. This allows the 2-3 stem structure to form, and interferes with the formation of the termination ~~loop~~ stem structure.

(11) RNA is alkali labile (unstable at pH 10) but DNA is not. [5 points]

Ribonucleotides contain a 2'-OH group which is absent in deoxyribonucleotides. Alkali induces deprotonation of the 2'-OH group facilitating its nucleophilic attack on the adjacent phosphorous atom, thereby cleaving the RNA backbone.

(12) Primers to a sequence that shows two-fold rotational symmetry (palindromes) cannot be used for Sanger DNA sequencing, site-directed mutagenesis, or PCR. [5 points]

These primers form self-complementary stems. They base-pair with themselves and not with the template. These techniques (DNA sequencing, site mutagenesis, & PCR) all require the DNA polymerase to extend the primer. But if the primer does not hybridize to the template, this cannot be done.

(13) RNA polymerase holoenzyme, when added to a non-supercoiled plasmid *in vitro*, causes the DNA to become supercoiled? [4 points]

RNA polymerase unwinds the DNA as it forms the open complex. Since the linking number of the DNA is unchanged, the untwisting of the DNA requires the formation of a supercoil.

Are the supercoils positive or negative? Why? [2 points]

positive.
The DNA is "overwound" since it must be untwisted to accomodate RNA polymerase.

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Explain briefly why (continued):

(14) *E. coli* strain K degrades (restricts) plasmid DNA isolated from strain B, even though strain B is known to contain a DNA methylating enzyme. [5 points]

Strain B contains a methylating enzyme with a different sequence specificity than the methylating activity of strain K. Thus, the strain K restriction enzyme (EcoK), recognizes ~~the~~ its unmethylated recognition sequence and cuts the DNA.

(15) Most transposable elements encode transposase genes with promoters that deviate from the consensus sequence of all known promoters. [5 points]

Promoters that deviate from the consensus are poor promoters. Transposable elements create mutations every time they transpose. It is important for transposable elements to keep the transposition rate low so they do not kill their hosts.

(16) Laboratories that amplify ancient DNA by PCR (Polymerase Chain Reaction) usually place a very strong ultraviolet lamp over the work site, but not over their samples. [5 points]

Contamination of samples is a problem with PCR. U.V. causes thymine dimers to form in the DNA of airborne contaminants. DNA containing thymine dimers cannot be amplified by PCR. Ancient DNA is badly degraded and trace amounts of undegraded contaminating DNA can easily dominate the reaction.

(17) The synthesis of new flagellin protein stops when flagella assembly is completed. [5 points]

Flagellin synthesis requires σ^F for its synthesis, but FlgM is an anti σ^F sigma factor. While flagella are being synthesized, FlgM is exported from the cell by passing through the hollow flagella lumin to the outside. On completion of the flagella, the ~~ta~~ lumin becomes blocked with flagellin and export of FlgM is stopped. FlgM then prevents σ^F from functioning.

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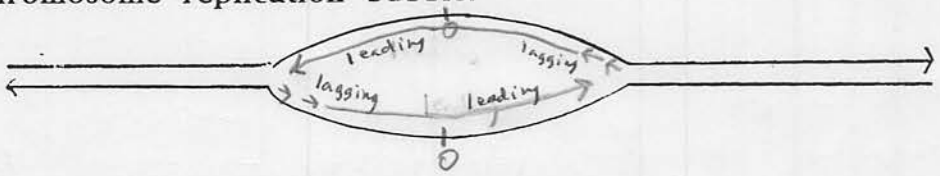
III. **The following diagram attempts to describe a newly initiated chromosome replication bubble.**



1. Label the origin on the diagram. [2 points]

2. Draw in the leading and lagging strands of DNA synthesis. Put arrowheads at the 3' ends and label the leading and lagging strands. [3 points]

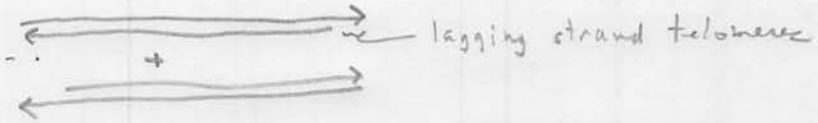
3. If this were a representation of a linear chromosome from a human liver cell:

(a) Describe the end replication problem (i.e., the problem with the replication of the chromosome ends). [3 points]

DNA polymerase requires a primer, which is usually RNA. At the chromosome ends, the RNA primer is degraded and there is no way to fill in the last portion of the lagging strand, since DNA polymerase can only synthesize DNA in the 5'→3' direction.

(b) What would be the condition of the chromosome after several (3 or 4) cycles of replication? Include a diagram of a replicated chromosome showing the ends which are effected by the end replication problem (label appropriately). [3 points]

The telomeres at the chromosome ends would get shorter by about 100 bases with each generation since both ends would not be able to replicate the lagging strand.



(c) How would telomerase solve the end replication problem? [3 points]

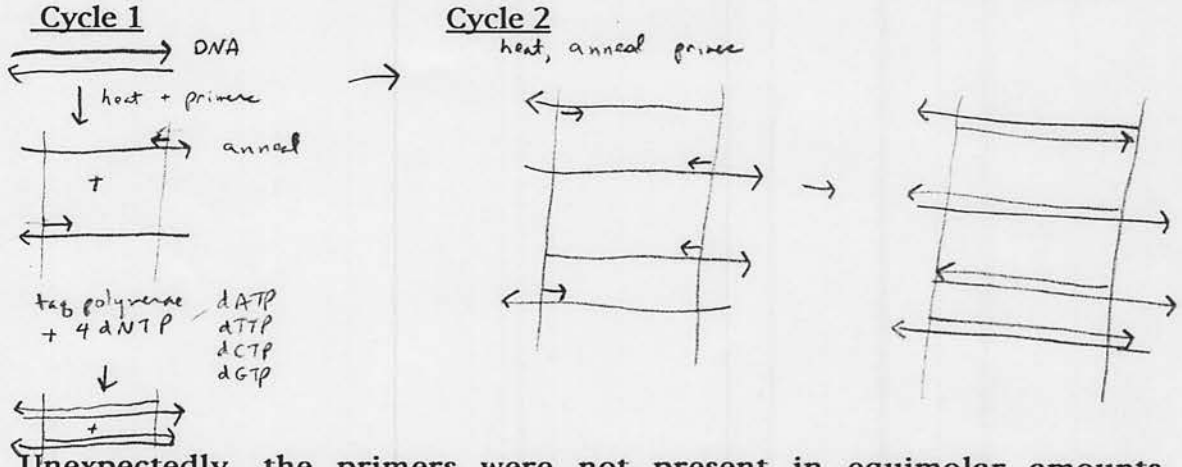
Telomerase is a reverse transcriptase which extends the telomeres of the leading strand using an RNA as a template (the RNA is basepair complementary to the telomere sequence and is part of the enzyme). This allows for the synthesis of a new primer for the lagging strand. Thus, telomerase allows telomeres to be extended.

(d) Why might oligonucleotides which were complementary (anti-sense) to the RNA component of telomerase be an effective potential drug for the inhibition of certain immortal cancer cells? [3 points]

Immortal cancer cells usually have a mutation which allows telomerase to be expressed in differentiated cells. Oligonucleotides which are complementary to the RNA component of telomerase can block its activity by hybridizing to the RNA component. This may cause:
a) destruction of RNA by forming an RNA-DNA target for RNase H
b) compete with telomeres for RNA
Blocking telomerase activity should limit the ability of some cancer cells to divide.

II. A scientist ordered two oligodeoxynucleotide primers for use in PCR.

(1) Draw a diagram to show how this technique will amplify DNA. Show two cycles of the reaction. Put an arrowhead at the 3' end of all DNA strands including primers. Include the names of all needed reagents (except buffers) and enzymes. [4 points]



(2) Unexpectedly, the primers were not present in equimolar amounts. One primer was present in 1/10 the amount of the other and would eventually be used up.

a. Describe the <u>principal</u> reaction product. [3 points]

Single stranded DNA corresponding to an extension of the more abundant primer. The sequence will start at the beginning of the primer and end at the end of the second primer

b. Suggest how this amplification (reaction product) might be useful. [3 points]

Source of single stranded DNA.
It can be used for DNA sequencing or site directed mutagenesis or as a probe for hybridization experiments.

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IV. A graduate student has discovered a mouse gene called "pudgy" that encodes a peptide hormone which, when lacking, results in very obese mice (pudgy mice).

1. Devise an experimental approach that could be used to identify the human homologue of "pudgy". [6 points]

A) Use mouse gene as a probe of genomic (human) Southern Blot → find the complementary band. If found, the band can be cut out of the gel and cloned.

or - mouse gene can be used to probe a cosmid or YAC human gene bank. The gene can be identified and cloned out.

or

2) Using the mouse gene sequence, a series of PCR probes can be made and used with human DNA. If an amplified gene is found, it can be used to probe a gene bank as described above

2. The student cloned the human "pudgy" gene in the plasmid pBR322 and inserted it into *E. coli* by transformation. However, no "pudgy" hormone could be detected in *E. coli*. In reviewing the failed experiment, the following problems to gene expression were considered. Can you help the student overcome these problems?

(1) Human genes contain introns as well as exons.

a. Why is this a problem? [1 point]

The coding region of the DNA is only found in the exon.
The presence of introns in the DNA would result in production of a meaningless peptide

b. Suggest a possible solution. [1 point]

1) Use cDNA made from mature (spliced) mRNA by reverse transcription of the RNA to DNA

or

2) recombinant DNA can be made using only the exon DNA: cut and paste method.

(2) Human promoters will not function in *E. coli*.

a. Why is this a problem? [1 point]

E. coli RNA polymerase requires the -10 and -35 regions of the promoter to transcribe a gene

b. Suggest a possible solution. [1 point]

Clone (insert) the gene behind a strong E. coli promoter such as lac or trp promoters.

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(3) A ribosome binding site may not be present on the mRNA.

a. Why is this a problem? [1 point]

Ribosomes will only translate proteins with a Shine-Delgarno sequence. This sequence is complementary to a short stretch of 16S rRNA

b. Suggest a possible solution. [1 point]

Clone the gene behind a strong E.coli ribosome binding site.

(4) The codon usage in the mRNA may not be appropriate.

a. Why is this a problem? [1 point]

Some codons are rarely used in E.coli and the corresponding tRNAs are not abundant. This may cause the ribosome to translate the gene very slowly.

b. Suggest a possible solution. [1 point]

Site directed mutagenesis can be used to change the rare codons to frequently used ones.

(5) The hormone may require post-translational modifications.

a. Why is this a problem? [1 point]

E.coli does not encode the proteases and other modification enzymes present in eukaryotic cells.

b. Suggest a possible solution. [1 point]

a) Incubate the precursor protein with extracts of human cells.

b) problem may not be possible to correct using an E.coli expression system. You may need to reclone the gene in a eukaryotic expression system. Try preparing a transgenic goat or sheep. It may be possible to express the gene so that it is secreted in the milk.

Name _____

III. (6 points) Compare and contrast the regulation of β-galactosidase production in *E. coli* by catabolite repression and the regulation of ovalbumin production by chick oviduct cells in response to β-estradiol (a steroid hormone). Please be concise and exclude characteristics that are negative for both or just plain trivial (for example, do not say "both are not found in Berkeley").

Similarities

(1) both are positive regulatory mechanisms

(2) both use small molecules (hormones) to turn on genes

(3) both work at the transcriptional level

(4) both hormones work on many genes

Differences

(1) sites of action
cAMP – works at promoter
β-estradiol – works at enhancer

(2) cAMP binds to CRP protein
β-estradiol binds to steroid receptor

(3) Catabolite repression is effected by glucose conc. ovalbumin production is not.

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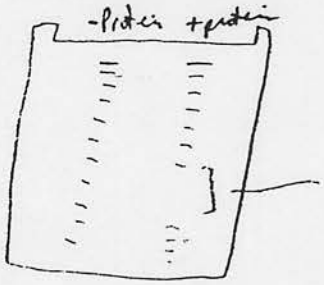
IV. Please outline in the space provided the best experimental evidence (you may devise your own as long as it is feasible) that would support the following conclusions. Be brief but complete.

1. (8 points) A newly isolated DNA binding protein (transcription factor) recognizes and binds to a specific 60 nucleotide region of DNA which lies 100 nucleotides upstream from the transcription start site (which is known).

The site of binding of a protein to DNA is best studied using a DNase footprinting experiment.

Mix 5'-labeled DNA + DNA binding protein

denature DNA and run on gel

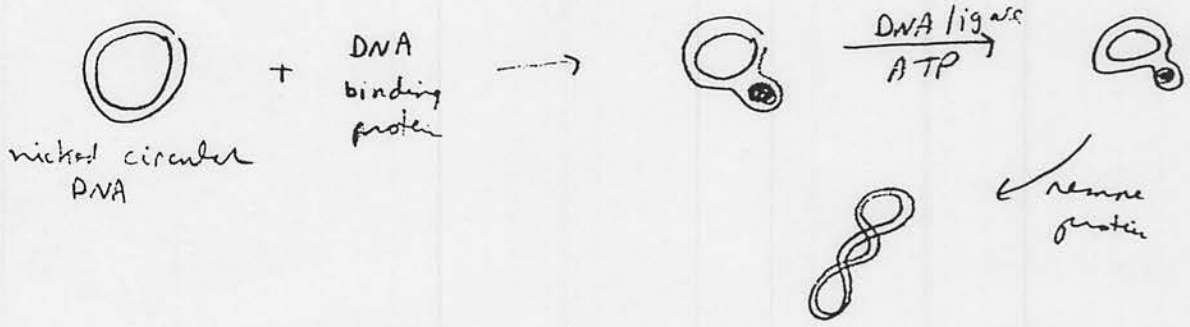


no protein band

footprint will appear where the DNA was protected from DNase action.

This can be aligned with the known sequence

2. (8 points) This DNA binding protein unwinds the DNA 720° (2 turns) when it binds to the DNA.



nicked circular DNA

two turns of helix should cause 2 supercoils to form because the DNA is underwound.

examin by electron microscopy

3. (8 points) Phage Q x 174 contains genes which overlap different translational reading frames.

1. sequence the DNA
open reading frames with AUG codons and Shine-Dalgarno sequences show overlapping reading frames

2. Mutations which alter 1 nucleotide can cause mutations in 2 proteins.