Restriction enzymes recognize DNA sequences that have 2-fold symmetry. The following DNA contains at least 5 restriction enzyme recognition sites. Find and draw a box around 4 possible recognition sequences that are either 4 or 6 base pairs in length. [You may also indicate the potential recognition sequences by writing them out, using brackets, etc]

```
5'- GAATTCCGCGCTTTGGATCCAT[GGCC]ATGGCCATT- 3'
3'- CTTAAGGGCGCGAAACCTAGGTA[CGGG]TACCCGGTAA - 5'
```

[8 pts] Shown below are the DNA sequences recognized by the enzymes \textit{Bam} HI, \textit{Hha} I, and \textit{Hae} III.

\textbf{Enzymes and cleavage sites:}

<table>
<thead>
<tr>
<th>\textit{Bam} HI</th>
<th>\textit{Hha} I</th>
<th>\textit{Hae} III</th>
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</thead>
<tbody>
<tr>
<td>5'-G↓GATCC-3'</td>
<td>5'-GCG↓C-3'</td>
<td>5'-GG↓CC-3'</td>
</tr>
<tr>
<td>3'-CCTAG↑G-5'</td>
<td>3'-C↑GCG-5'</td>
<td>3'-CC↑GG-5'</td>
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</table>

Answers: highlighted in yellow and in [brackets]

Indicate the fragments obtained from the given DNA sequence after cleavage with these enzymes. [Arrows or boxes work well to mark the sequences to indicate the cuts and fragments obtained by each enzyme. You DON’T need to rewrite the sequences of the fragments]

a) \textit{Bam} HI fragments [2 pts]

```
5'-GAATTCCGCGCTTTGGATCCATGGCCATT-3'
3'-CTTAAGGGCGCGAAACCTAGGTACCCGGTAA-5'
```

b) \textit{Hha} I fragments [2 pts]

```
5'-GAATTCCGCGCTTTGGATCCATGGCCATT-3'
3'-CTTAAGGGCGCGAAACCTAGGTACCCGGTAA-5'
```

c) \textit{Hae} III fragments [4 pts]

```
5'-GAATTCCGCGCTTTGGATCCATGGCCATT-3'
3'-CTTAAGGGCGCGAAACCTAGGTACCCGGTAA-5'
```
[2 pts] The biological role of restriction enzymes in bacteria is to:

A) repair DNA.
B) induce DNA supercoiling.
C) Cut and inactivate foreign RNA.
D) Cut and inactivate foreign DNA.
E) Clone DNA fragments.

[3 pts] How do DNA fragments of various sizes separate on agarose and acrylamide gels?

Answer: Electrophoresis is used to separate DNA. Fragments migrate according to size, with larger fragments migrating more slowly than smaller fragments.

[2 pts] What governs the choice of using agarose or acrylamide to separate pieces of DNA?

Answer: Agarose gels are used for larger fragments. Polyacrylamide is used for shorter fragments, and can resolve fragments differing by as little as one base.

[2 pts] How can DNA molecules be detected on either kind of gel?

Answer: Several techniques for visualization can be used, including fluorescence of ethidium bromide stain, radiography, or other fluorescent tags.

[5 pts] What are some properties of a DNA probe that would allow it to be used to identify a specific DNA restriction fragment on a Southern blot?

Answer: A DNA probe must have a complementary base sequence that allows it to base pair [hybridize] with one strand of the restriction fragment DNA. The probe must be “labeled” to permit detection of the restriction fragment. Probes can be small oligonucleotides (~20 bases long) or longer DNA fragments.

[2 pts] Starting with 2 copies of a double-stranded DNA, how many copies of that DNA will there be after 3 repetitive cycles of PCR (polymerase chain reaction)? 16 copies.
[6 pts] (Circle the correct answers) [2 pts each]

Chain termination DNA sequencing is used to solve full genome DNA sequences. The DNA sequencing method succeeds because there is no (3'-H, 2'-H, 3'-OH, or 2'-OH) on the last nucleotide added to a growing DNA chain.

DNA sequencing is possible because gel electrophoresis can be used to discriminate between (DNA labeled with different isotopes, DNA differing in length by only one base, different DNA restriction enzyme fragments).

Current automated detection of fluorescence-tagged DNA fragments permits DNA sequence determination only if all DNA fragments ending in each of the four bases (are randomly labeled with fluorescent molecules, produce fluorescent signals of similar strength, are present in increasing amounts as the DNA fragment length increases).

[8 pts] ANSWER TRUE OR FALSE

**False** Chemical synthesis of DNA oligonucleotides proceeds from the 5' to the 3' end.

**True** If a foreign gene is inserted into a plasmid's tetracycline antibiotic resistance gene, host cells containing the recombinant DNA plasmid will not grow in the presence of tetracycline.

**False** An elephant hemoglobin gene can be expressed as a translatable mRNA in *E.coli* cells

**True** Chemical synthesis can produce complex mixtures of small DNA molecules that all are the same length but which differ in base sequence.

[2 pts] Name/Identify two ways PCR can be used in medical diagnosis.

**Answer:** PCR can be used to identify the presence of infecting bacteria or viruses; can detect if certain mutations, such as those found in cancer cells, are present; and can be used as a diagnostic tool to investigate known gene mutations. [etc...]

[4pts] How is a single gene of interest identified on a plate containing many different gene library clones?

**Answer:** By using a probe specific for the DNA of interest, the clone can be identified. The probe is designed to hybridize to the DNA of the clone that has been transferred to a membrane. The probe is labeled with radioactivity or another tag so that it can be easily detected and the proper clone identified and selected from the original plate.
Multiple Choice Questions [2 pts each]

Why are methionine and tryptophan desired in a peptide sequence that is reverse-translated to design DNA probes from amino acid sequences?

A) Each has only one possible codon sequence.  
B) Met is the first amino acid in the protein.  
C) Both are used often in proteins.  
D) All of the above.  
E) None of the above.

To identify a gene with a "probe," to which strand of DNA must the probe be complementary?

A) only the coding strand  
B) only the template strand  
C) both strands  
D) either strand  
E) None of the above.

Genes can be inserted into eukaryotic cells by:

A) viruses.  
B) microinjection.  
C) liposomes.  
D) None of the above.  
E) All of the above.

Animals that harbor a foreign gene as a result of recombinant gene manipulation are called

A) transgenic.  
B) mutants.  
C) aliens.  
D) All of the above.  
E) None of the above.

The protein enzymes listed below all function in interactions with DNA. The binding of these proteins to DNA are either independent or dependent on the nucleotide sequence of the DNA with which they interact. From what you have learned about the principles of protein/DNA interactions, classify each protein's basis of interactions with DNA as:

\[ \text{d} \quad \text{(Dependent on the DNA nucleotide sequence) or} \]
\[ \text{i} \quad \text{(Independent of the DNA nucleotide sequence)} \]

Write \text{d} or \text{i} in the space provided

\text{d} \quad \text{Sal I restriction enzyme}  
\text{i} \quad \text{Deoxyribonuclease I}  
\text{i} \quad \text{DNA ligase}  
\text{d} \quad \text{Eco RV restriction enzyme}  
\text{i} \quad \text{Thermus aquaticus (Taq) DNA polymerase [for PCR]}
Circular double-stranded DNA from SV40 virus was isolated and subjected to agarose gel electrophoresis. The results are shown in lane A of the adjoining gel patterns. The length in base pairs of the DNA in bands 1 and 3 are the same.

[DNA migrates in this gel from top (-) to bottom (+)]

[2 pts] What property causes the 2 classes of DNA in Lane A to separate so far in agarose gel electrophoresis?

Answer: Bands 1 and 3 differ in topology, i.e., their state of supercoiling [open circular vs. supercoiled]

[2 pts] In Lane A, how do the DNA in bands 1 and 3 differ from each other?

Answer: The sizes are the same, but the DNA at the top (band 1) is relaxed, open circular and the DNA at the bottom (band 3) is highly supercoiled.

The same DNA sample used in lane A was then incubated with topoisomerase I for 5 minutes and then analyzed by gel electrophoresis (results shown in lane B).

[2 pts] In lane B, what is the relationship between the DNA bands 1 and 3 and those in the brackets [labeled 2]?

Answer: All bands (1-3) are topoisomers (all are the same size) that have different degrees of supercoiling. The DNA is highly supercoiled at 3 and relaxed at 1. Discrete intermediate forms are within the brackets (2).

Another sample of the same DNA used in Lane A was incubated with topoisomerase I for 30 min and again analyzed as shown in lane C.

[4 pts] In lane C, why is most of the DNA in slower moving forms [at band 1 and within bracket 2]?

Answer: The degree of supercoiling decreasing from band 3 to band 1 with increasing time in the presence of topoisomerase I. The DNA is becoming progressively more unwound, or relaxed, with less and less supercoiling, and thus slower moving on the gel, because the DNA becomes more like open circles which are retarded in the gel matrix, relative to the supercoiled form of this DNA.
[6 pts] Name the 3 distinct regions/main features of a general cloning plasmid, (i.e. such as pUC18 or pBR322) indicated below as A, B and C (order does not matter):

**Answer**

A: cloning site – restriction enzyme cleavage site  
B: antibiotic resistance gene for selection  
C: origin of replication

[5 pts] The figure below shows the process of creating a cDNA Library: In the numbered boxes, fill in the names of the components found within the box.

**Answers:**

1) Oligo(T) primer  
2) Poly(A) tail  
3) cDNA  
4) mRNA  
5) Double-stranded cDNA

[6 pts] ANSWER TRUE OR FALSE

**True**  Proteins whose binding to DNA is dependent on the specific base sequence of the DNA generally form hydrogen bonds between amino acids and functional groups located in the major groove of the DNA B helix.

**True**  The exact 3-dimensional structure of DNA is strictly dependent on its base sequence.

**True**  Proteins whose binding to DNA is not dependent on specific base sequences generally form ionic bonds between (+) charged amino acids and (-) charges on the phosphate backbone of the DNA B helix.
[8 pts] SUSHI FRAUD!! NAME THAT SPECIES!! I love sushi, but I suspect that some Tucson restaurants are dishonest in naming the type of fish that they call “fatty tuna”, a sushi delicacy. I have collected samples of this sushi from several of these renegade restaurants and want you to help me prove whether these samples are authentic “fatty tuna”?

a) Name two recombinant DNA techniques that you can use to identify the fish species to which these samples belong.

b) How can these techniques be used to identify the species of fish corresponding to each fish sample? Describe how you would do this in a few sentences [or a list of steps you will take]. Credit will be given for proposing any procedure that is reasonable and correct [i.e., will actually work].

Answer: PCR, DNA sequencing, RFLPs [restriction enzyme cuts of genes that show a difference between fish species], do fish BarCode of Life analysis, etc. [Too many possibilities to list.]

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EXTRA CREDIT

[5 pts] Write the DNA sequence that can be deduced from the DNA sequencing gel fragment pattern shown below: [full credit only if written in correct chain polarity]

Answer: 5’ - ATGGGCATACTCTGAA - 3’

A G T C

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(+e) electrode