For each item, your tasks are highlighted in **bold face**.

I. Interpretation (35 points total)

A. Translation

In vitro translation of four RNAs was studied. Each RNA had a 5’ cap structure, a 3’ poly A tail and at least one open reading frame. RNA 1 had just the A open reading frame and RNA 2 had only the B open reading frame. RNA 3 had A as the 5’-most open reading frame, followed by the B open reading frame. RNA 4 was like RNA 3, but with the order of open reading frames reversed.

![RNA structures with 35 and 42 kDa products](image)

In vitro translation of RNAs 1 and 3 and analysis of the polypeptide products revealed a product of 35 kDa. Similar translation of RNAs 2 and 4 gave a 42 kDa polypeptide. None of the translations yielded two polypeptides.

**Assignment:**

What generalization about initiation of protein synthesis in eukaryotes does this result support (5 points)?
B. Organelle genetics
A gene encoding resistance to an antibiotic was provided with a promoter active in chloroplasts. The construct was biolistically shot into cultured plant cells. Cells resistant to low levels of the antibiotic were selected. Upon successive passage, the cells gradually became resistant to higher concentrations of the antibiotic.

**Assignment:**
Suggest an explanation for the gradual change in resistance level, consistent with present understanding of plastid genetics (6 points).

C. Transcription
A 40 bp DNA corresponding in sequence to a section in the middle of the frog 5S rDNA gene was synthesized and labeled. The labeled DNA was added to tubes containing the general RNA polymerase III transcription factors TFIIIA or TFIIIB or both factors. The tube contents were analyzed by gel electrophoresis with the following result.

![Diagram showing mobility and additions of TFIIA and TFIIIB](image)

**Assignment:**
What does the result suggest about the location of the promoter for 5S rRNA transcription (2 points) ?

**Assignment:**
What can be inferred about the role of TFIIIA in transcription (4 points)?
D. Site-specific recombination

The target site for the yeast FLP recombinase consists of inverted repeats flanking an 8 bp spacer sequence. FLP catalyzes inversion of a section of the 2 micron plasmid. When the 8 bp sequence is altered so that it is symmetrical (a palindrome), FLP catalyzes deletions as well as inversions.

Assignment:
Explain the importance of the spacer sequence for the outcome of site-specific recombination (6 points).

E. DNA repair

Mammalian cells in culture are UV-irradiated, creating thymine dimers in the DNA. After various times of incubation, DNA is prepared and one aliquot treated with T4 endonuclease (this enzyme nicks DNA at positions of thymine dimers on the same strand as the dimers). The DNA is restricted and denatured. The single strands are spread out by electrophoresis and Southern blotted. The blot is probed by hybridization with strand-specific probes (“Transcribed” and “Non-transcribed”) for the dihydrofolate reductase gene.

```
  0  3  6  9 h post UV
- + - + - + - + T4 endo
--- ----- ~~~ --- Transcribed
--- --- --- --- Non-transcribed
```

The diagram shows an idealized result. Probes for the transcribed strand reveal development of resistance of the DNA to T4 endonuclease with time of culture. Similar results are obtained when transcribed and non-transcribed strands of other genes are probed.

Assignment:
What relationship between transcription and DNA repair is implied by these results (6 points)?
F. Transcription regulation
Nuclei isolated from duck reticulocytes (globin-synthesizing cells) and duck oviduct cells (synthesizing ovalbumin) were treated with low concentrations of DNase that release only 10% of the total DNA from the nuclei. After the DNase treatment, the nuclei were recovered by sedimentation. Their content of globin and ovalbumin genes was measured. There was a lot more ovalbumin DNA in the treated reticulocyte nuclei than in the treated oviduct nuclei. Conversely, there was a lot less globin DNA in the treated reticulocyte nuclei than in the treated oviduct nuclei. Untreated nuclei from the two sources had equivalent amounts of both DNAs.

Assignment:
Describe the current view of the relationship between chromatin structure and gene activity that this result is consistent with (6 points).

II. Basis for principles (30 points)

A. Principle:
The nucleotide sequence of a DNA and the universal genetic code are insufficient to reliably deduce the amino acid sequence of a protein.

Assignment:
Give one reason that the above statement is thought to be true. (6 points)

Assignment:
Give another reason that the above statement is thought to be true (substantially different from the first). (6 points)
B. Meiotic DNA recombination creates regions of heteroduplex DNA.

**Assignment:**
Describe one observation consistent with this generalization. (6 points)

C. Telomeres stabilize chromosome ends.

**Assignment:**
Describe one observation which supports this conclusion. (6 points)

D. Some introns in RNA are removed using as catalyst the RNA in which they reside.

**Assignment:**
Describe one experimental observation that supports this conclusion. (6 points)
III. Experimentation (35 points)

A. The Bz gene, responsible for pigmentation in the aleurone layer of maize kernels, was isolated as a lambda DNA clone from each of two maize plants. One plant was phenotypically wild-type. The other had an unstable mutation at the Bz locus. Kernels were often variegated.

The restriction maps for two enzymes, \textit{MgeI} and \textit{UkmlII} of the two clones were compared. They were identical except that a 0.3 kbp \textit{MgeI} fragment from the wild-type plant was replaced by a 7 kbp \textit{MgeI} fragment in the DNA from the mutant plant.

You hypothesize that the extra size of the \textit{MgeI} fragment and the unstable mutation were due to the insertion of a transposable element. You would like to get support of your hypothesis from DNA sequence analysis as soon as possible. Recall that DNA sequence determinations usually yield 400 to 800 nt of information at each run.

\textbf{Assignment:}

What parts of which DNAs would you target for sequencing (5 points)?

What nucleotide sequence features would you expect to find if your hypothesis was correct (8 points)?
B. A single-stranded DNA molecule coated with recA protein will, in the presence of ATP, make a complex with a double-stranded circular DNA (no free ends). The complex will be plectonemic if the single-stranded DNA has the same nucleotide sequence as part (or all) of the circular DNA. Otherwise, it will be paranemic.

**Assignment:**
Describe an experiment to test whether the sequence shared with the circular DNA must be at the 5’ end, the 3’ end or the middle of the single-stranded DNA (10 points).

C. Gene isolation
A series of genes are to be isolated.

**Assignment:**
Match each gene (on the left) with the method (from the right column) most likely to be used in its isolation (12 points)

<table>
<thead>
<tr>
<th>A gene from papaya for an enzyme that has been substantially purified. ____</th>
<th>1. Inverse PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>A gene of rice whose mutation results in a visible phenotype but whose biochemical basis is unknown. ____</td>
<td>2. Map-based cloning</td>
</tr>
<tr>
<td>A gene from chimpanzees equivalent to the isolated human alcohol dehydrogenase gene. ____</td>
<td>3. Antibody screening of plaques of a cDNA expression library</td>
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<tr>
<td></td>
<td>5. Complementation screening</td>
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