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

I. Basis for principles (30 points)

   A. Principle:
      Ends of DNA at chromosome ends are different from DNA ends not at chromosome ends

      **Assignment:**
      Describe experimental observations that support the above statement. (6 points)

   B. Principle:
      DNA genomes may be programmed to rearrange themselves.

      **Assignment:**
      Describe an example that supports the above statement. (6 points)

   C. Principle:
      Molecular mechanisms for attachment of a chromosome's DNA to the mitotic spindle have not been conserved in evolution.

      **Assignment:**
      Describe experimental observations that support the above statement. (6 points)
D. Principle:
Mitochondrial genomes of vertebrates are maternally inherited.

Assignment:
Describe an experimental observation that supports the above statement. (6 points)

E. Principle:
Recombination is initiated at specific sites along the genome.

Assignment:
Describe an experimental observation that supports the above statement. (6 points)
II. Experimentation (35 points)

A. DNA repair. Facts and observations:
   1. Multiple systems for repair of damage to DNA exist in most cells.
   2. You are studying a novel modified base that could arise by reaction of DNA with a new chemical.
   3. You have made a double-stranded circular DNA containing a single unit of this novel base at a known position in the sequence of the DNA. You can also make this molecule with specific nucleotides (or their bases) labeled.
   4. When transformed into target cells, the apparent DNA damage is repaired.
   5. Extracts of these cells are known to perform reactions of nucleotide excision repair (NER) and of base excision repair (BER).

Assignment:
   Describe a test of whether the modified nucleotide is removed by NER. (10 points)

Describe the expected results of your test and how you would interpret them. (5 points)
B. Centromeres. Facts and observations:
   1. You have cloned a sequence that you think functions as a centromere in yeast.
   2. You have available all molecular biological tools of a yeast molecular biologist.

Assignment:
   Devise a test of whether the sequence functions as a centromere in yeast and describe the test. (7 points)

Describe the expected results of your test and how you would interpret them. (3 points)

B. Mitochondria. Facts and observations:
   1. By density gradient centrifugation, you isolated a “satellite” DNA, a DNA whose average buoyant density is quite different from the bulk of DNA of the organism you are studying.
   2. You suspect that this DNA is mitochondrial DNA rather than highly repeated nuclear DNA.

Assignment:
   Devise a test of your suspicion and describe the test. (7 points)

Describe the expected results of your test and how you would interpret them. (3 points)
III. Interpretation (35 points total)

The following completely hypothetical narrative is based on a compilation of molecular genetic phenomena known in a variety of organisms. Questions requiring short answers are interspersed with the narrative in bold face. Diagrams are included to help you follow the narrative.

Wizards are tiny multicellular organisms that can be cultured readily in liquid media and on agar plates. They have been well studied genetically. When a culture of wizards was treated with a peptide called magic, many mutant wizards were produced. Several of the mutants had phenotypes identical to those of wizards with mutations in known (= isolated and sequenced) genes. Of these, fifteen mutants, representing independent mutations, were analyzed. The mutated genes were isolated and their nucleotide sequences determined. In each case, the mutated gene contained from 2 to 5 kbp of sequence additional to that present in the wild type gene. In each case, identical 24 bp sequences were found at the ends of the extra DNA, in inverted orientation relative to one another. Adjacent to these extra DNA sequences, the identical 5 bp of the wild type gene were found on each side.

![Diagram](image)

A. To what category of genetic sequences do these elements belong? (3 points)

Of the 15 extra sequences, five were identical and of 5 kbp. They were named Oz elements. Oz elements were predicted from the sequence to encode a single protein. Five others were less than 5 kbp in length and were deleted versions of Oz. They were called Topeka elements. The remaining five were of a variety of lengths and, other than the inverted repeat termini, contained sequences unrelated to Oz. They were called Witchita elements.
B. What is the probable relationship between Oz and Topeka elements? (3 points)

C. What is a reasonable hypothesis for the evolutionary origin of Witchita elements? (3 points)

Oz sequences were used to probe a cDNA expression library made from polyA (+) RNA of magic-induced wizards. A cDNA clone was isolated that in a bacterial plasmid expression vector caused bacteria to make a polypeptide, called Em. Em, isolated from bacteria, was used to immunize rabbits. An antiserum, anti-Em, was obtained. Anti-Em was used to probe western blots of proteins extracted from uninduced wild-type wizards and from magic-induced wizards and separated by SDS-PAGE. A polypeptide band of 50 kDa was detected in the uninduced sample. The magic-induced sample had a band of 60 kDa.
D. List four diverse plausible reasons for the different apparent sizes of the Em polypeptide in magic-induced and uninduced cultures, based on your knowledge of causes of the production of multiple polypeptides from a single gene in other systems. (5 points)
Poly A (+) RNA was isolated from non-induced and magic-induced wizard cultures. The RNA was separated by gel electrophoresis, and northern blotted to a nylon membrane. The blot was probed with the Em cDNA. A single RNA band of 2.4 knt was detected in the sample from non-induced wizards. A single 2.1 knt band characterized the sample from magic-induced wizards.

E. List two plausible reasons for the different apparent sizes of the Em mRNA in magic-induced and uninduced cultures, based on your knowledge of gene expression in eucaryotes. (5 points)
The cloned Em cDNA was used to probe a cDNA library prepared from polyA (+) RNA of non-induced wizards. Positive cDNA clones had their nucleotide sequences determined. The sequences had 0.3 kbp of sequence not present in the previously isolated magic-induced Em cDNA. The extra sequence ended in an AG dinucleotide. Relative to the Oz element sequence, both the induced and non-induced cDNAs were missing a 0.5 kbp sequence that began with a GT dinucleotide and ended in an AG dinucleotide. This sequence in Oz was immediately upstream of the 0.3 kbp sequence missing in the magic-induced cDNA.

F. Explain the generation of each the 2.1 and 2.4 knt Em mRNAs from Oz DNA in wizards. (3 points)
Differential display RT-PCR was used to identify and isolate cDNA sequences that were specific to magic-induced wizards. The differential display bands in the two samples were almost identical. The sample from magic-induced wizards did contain one novel band. This was isolated and used to obtain a full-length cDNA clone from the cDNA library from magic-induced mRNA. The nucleotide sequence of the clone was determined. A polypeptide of 34 kDa could be encoded by the sequence. It was called Dorothy. Dorothy was similar in sequence to that of the product of the sxl (sex lethal) gene of Drosophila.

G. What is the probable role of Dorothy? What will it likely interact with? (3 points)
Dorothy cDNA was used as a probe to isolate the Dorothy gene from a genomic library. A 1 kbp sequence immediately upstream of the Dorothy mRNA transcription start site on the gene was ligated to a cDNA encoding an easily assayable enzyme, Toto, in a vector that could shuttle between E. coli and wizard cells. Magic peptide treatment of wizard cells transformed with the construct resulted in synthesis of Toto enzyme activity. Mutations were made at selected places in the 1 kbp sequence. Only mutations in an octanucleotide sequence reduced magic-induced Toto activity.

H. List two other cDNAs that could have been used in place of Toto and give the name used to describe such sequences. (5 points)
A mobility shift assay was used to isolate a protein, called munchkin, that bound specifically to the octanucleotide sequence. Binding of munchkin to the DNA was dependent on the presence of the magic peptide in the assay mixture.

| magic | - | - | + | + |
| munchkin | - | + | + | - |
| oligo | + | + | + | + |

I. What is the likely function of munchkin? (5 points)

Click your heels twice, think of home and have a happy holiday!