For each item, your tasks are highlighted in **bold face**. Please ask for clarification if you do not understand a question!

I. Interpretation (35 points total)

   A. Facts and observations:
   Recall that as a result of DNA replication of linear molecules (such as a chromosome), each end of the parent should generate one blunt-ended double-helical daughter (parental 5' end strand) and one 3' single-strand overhang double-helical daughter (parental 3' end strand). We will call the former the "leading end" and the latter the "lagging end". Also recall that immediately after DNA replication, duplicated double strands are held together as sister chromatids until mitosis.

   TRF2 is a protein found at the junction (D-loop) regions of human telomere lariats. A line of cells has been constructed by addition of an inducible mutant TRF2 gene to wild type cells. The mutant gene, when induced, produces a dominant negative phenotype that includes chromosome end fusion and anaphase bridges.

   **Assignment:**
   What hypothesis for the function of telomeres does the mutant phenotype support? (7 points)

   **Assignment:**
   What is the likely role of TRF2 in the telomere function you identified above? (6 points).
End fusions were only detected between replicated chromosomes. They were not seen between sister chromatids. The investigators devised a method to distinguish leading from lagging ends. They used the method to test which ends bound to which in the chromosome fusions. They found exclusively leading-end-leading end fusions.

**Assignment:**

Give a plausible hypothesis for the structural requirements for the ends joined by the machinery that joins the ends. (6 points).

**Assignment:**

What may the normal function of the machinery that joins the ends be? (3 points).

**Assignment:**

Evaluate the consistency of the structural requirements you hypothesize with the absence of sister chromatid fusions. (3 points).

**B. Facts and observations:**

A microsatellite repeat, (GCCAC)\(n\), probed (by hybridization) fractions of a CsCl density gradient distribution of DNA preparations from a eukaryote. When the DNA preparation used had been sheared to an average size of 300 bp, the probe detected DNA of a density higher than that of the main band of DNA. When the DNA preparation used had been sheared to an average size of 4,000 bp the probe detected DNA of a density equivalent to that of the main band DNA.
Assignments:
Why does the microsatellite repeat appear to have densities that depend on the length of the DNA analyzed? (10 points)
II. Basis for principles (30 points)

A. Principle:
The molecular genetic tools of
- DNA isolation,
- molecular cloning,
- Southern blotting-hybridization,
- restriction enzyme cleavage,
- specific sequence amplification by polymerase chain reaction, and
- gel electrophoresis
can be combined in various ways to detect molecular genetic polymorphisms in populations.

Assignment:
Describe two techniques that illustrate the above assertion. A complete answer will include more than the names of techniques. It will include a description or diagram of the steps involved in carrying the technique out. (10 points)

B. Principle:
Transposition of elements such as *gypsy* in yeast proceeds through a mRNA intermediate.

Assignment:
Describe an experiment that supports the above assertion. (10 points)

C. Principle:
Centromere sequences are not phylogenetically conserved.

Assignment:
Describe observations that support the above assertion. (10 points)
III. Experimentation (35 points)

Using microarray technology, investigators found that the expression of 20 genes in a mammalian cell line was induced many fold by the addition of cadmium ion to the growth medium. Since the line was derived from an organism whose genome has been completely sequenced, the investigators used the consensus sequence approach and identified a heptanucleotide sequence GATACAC present between 100 and 200 bp upstream of the transcription start sites of all 20 of these genes.

This observation led to two hypotheses:
Hypothesis 1: GATACAC is the target for binding of a factor present in its binding-active form in the presence of cadmium ion but not in its absence.

Assignment:
Design and describe an experiment to test hypothesis 1. (10 points).
You have available all the tools of molecular biology, but you must specify which you are using in your experiment.

Describe (and/or diagram) the results expected if the hypothesis is correct. (4 points)

Describe (and/or diagram) the results expected if the hypothesis is not correct (4 points).
Hypothesis 2: Each bp of the GATAACAC sequence is required for the induction of high levels of gene expression by cadmium ion in the cell line being studied.

Assignment:
Design and describe an experiment (or experiments) to test hypothesis 2. (11 points). You have available all the tools of molecular biology, but you must specify which you are using in your experiment. If you use any DNA constructs in your strategy, be sure to describe these fully as to sources and orientations of their components.

Describe (and/or diagram) the results expected if the hypothesis is correct. (3 points)

Describe (and/or diagram) the results expected if the hypothesis is not correct (3 points).