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

I. Basis for principle (40 points)

**Principle:**
Ends of DNA molecules in eukaryotic nuclear chromosomes are active in many processes unless they have special structures or features.

**Assignment:**
Write a short essay giving examples of observations that support the assertion. In your essay, you should include at least one observation from each of the following aspects of molecular genetics: 1) cell cycle regulation, 2) chromosome stability, 3) chromosomal DNA sequence, 4) meiotic recombination. (Use the back of this page if you need more space. Be sure to describe the observation before giving its interpretation.)

*Ends of DNA molecules are special. They are formed in the process of replication and during some types of DNA damage and as intermediates in DNA repair. They are important for initiating recombination. Perhaps because they are necessary consequence of replication and are often present after DNA damage prior to repair, eukaryotes have evolved a process of sensing free DNA ends. This sensing leads to prevention of cells entering the mitotic part of the cell cycle. The clearest demonstration of this is the arrest of the cell cycle in yeast resulting from the induction of the HO endonuclease gene supplied under control of the gal promoter. Natural DNA ends at telomere form unique G= quartet structure, dictated by their DNA sequences and are targets of binding telomere specific proteins. One or more of these properties prevents these ends from signaling mitotic arrest and participation in recombination. The latter observation is important for chromosome stability. Instability is obvious from the consequences of breaking chromosome at Ds in maize: fusion of broken ends. Genes essential for and appearing at initiation of meiotic recombination include nuclease that ceate ends used in initiating recombination.*

II. Interpretation (30 points total)

A. Facts and observations:
A pair of allele-specific PCRs has been devised to distinguish between mitochondrial DNAs of two mouse strains (A and B) and is used in investigation of cross-strain *in vitro* fertilization. Zygotes were created by adding A strain sperm to B strain oocytes. DNA was extracted from an aliquot of purified
zygotes as soon as feasible after fertilization. Its use in allele-specific PCR revealed the presence of amplified DNA bands after electrophoresis characteristic of each A and B mitochondrial DNA. After incubation of the remaining zygotes for a period of time, extraction and PCR was repeated. Only the B-characteristic band was observed.

Assignment:
Based on this result, what kind of organelle inheritance (maternal, paternal, biparental) do you expect in mice? (5 points)

Maternal

Assignment:
Present a hypothesis (or hypotheses) that explains the difference between the results of the PCR of early isolated DNA and the results of the PCR of later isolated DNA. (10 points)

Paternal organelles are not excluded during fertilization. Rather they are actively destroyed after cytoplasmic fusion.

B. Facts and observations:
The storage loci of yeast mating type information, HML and HMR, are not normally active in transcription. The silent state of these genes is stably inherited. Thus, only the MAT locus information is transcribed. Yeast mutants have, however, been identified that allow these loci to become active in transcription in subsequent generations. The loci are of two types. HMR-E is a 140bp DNA sequence element necessary for silencing of transcription of HML and HMR. The second type of loci encode protein factors. A series of Sir (silent information regulator) genes encode such proteins needed for the silencing. Activities of two of the proteins have been identified as:

• Sir2p is a histone deacetylase.
• Sir4p binds to deacetylated histone tails and to Sir2p.

Assignment:
Present a model that can explain how Sir2p and Sir4p could lead to inactivation of a large stretch of chromosome for transcription. (15 points)

Sir2p deacetylates histone tails on one nucleosome. The deacetylated tail serves as a binding site for Sir4p. Sir4p (bound to the nucleosome) now serves as binding site for Sir2p which is bridge to the neighbor nucleosome, deacetylating its tails.

III. Experimentation (30 points)
A. For this task, your favorite organism (YFO) is a unicellular eukaryote whose genome sequence is not yet known. Its genetic information is present on four
large linear chromosomes. Your assignment is to investigate the sequence of the ends of these chromosomes without sequencing the whole genome. You have isolated total DNA from YFO. You have available leu2- (mutant) yeast cells and the small stable linear yeast plasmid DNA, of known nucleotide sequence, produced by Nobelists Blackburn and Szostak, bearing a LEU2 (wild type) gene. You also have genetic engineering tools such as restriction endonucleases and DNA ligases and capability for growing and transforming yeast.

Assignment:
Outline a protocol that would produce a DNA preparation enriched for sequences from the ends of YFOs (10 points).

The plasmid available has the structure:

TELL -------LEU2--------TELR

Digest it with a restriction endonuclease (if possible) to remove one end only to give

--------LEU2--------TELR

From YFO DNA prepare a digest with the same restriction enzyme.
Mix the fragments with the prepared vector and ligate.
Transform leu2- cells. Select for growth in the absence of leucine.
Test transformants for ability to maintain the leu+ phenotype.

Assignment:
Describe how you would obtain the sequence of the ends. Begin your description with the product of the protocol you have just designed (5 points).

Isolate plasmid DNA. Design a primer from the end of the known vector, inside the restriction site use.
Use it in Sanger chain termination synthesis sequencing with transformant plasmid DNA as template. Sequence should terminat abruptly as the polymerase falls off the template.

B. You wish to test the hypothesis that a 1 kbp fragment of the YFO genome functions as an origin of replication in YFO cells using standard methods of molecular biology and/or molecular genetics.

Assignment:
Describe one approach to testing the hypothesis. There are multiple correct answers for this question. You need describe only one (10 points).

Multiple answers are possible. The expected one was:
2-D gel electrophoresis of restriction fragments of the DNA from dividing cell populations are made. The transferred DNA is probed with a specific DNA fragment using Southern blotting and hybridization.

Assignment:
Describe the expected results if the 1kbp fragment does function as an origin of replication. Describe the expected results if the 1 kbp fragment does not function as an origin (5 points).

The result will show a discontinuity in the pattern as the replication bubble conformation (two forks moving in opposite direction) bursts when one of the two forks reaches the end of the fragment.