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

I. Basis for principles (35 points)
   A. Principle:
      The genetic codes used by all organisms had a single evolutionary origin.
   
      **Assignment (9 points):**
      Describe the evidence supporting this assertion.

   B. Principle:
      Termination of transcription of protein coding genes plays only a minor role (if any) in eukaryotic gene expression.

      **Assignment (8 points):**
      Describe the evidence supporting this assertion.

   C. Principle:
      The DNA sequences that signal a place for DNA replication to initiate differ between organisms.

      **Assignment (9 points):**
      Describe the evidence supporting this assertion.
D. Principle:
Inversions and translocations are rare events in the chromosome evolution of organisms.

Assignment (9 points):
Describe the evidence supporting this assertion.

II. Interpretation (35 points total)
A. Facts and observations:
You have a genomic library from MFO. The inserts of several clones have had their sequences of nucleotides determined. One is of particular interest to you. You design primers to amplify a 500 bp segment of the insert sequence, using mRNA as template. Amplification is attempted by a reverse transcription- polymerase chain reaction procedure known to amplify well fragments of 2 kbp or less. No product is detected, even though positive controls included in the experiment (including template RNA transcribed in vitro from the DNA genomic clone and purified) did produce the expected product.

Assignment (10 points):
Provide a likely explanation for this result (several good ones are possible; choose only one).
B. Facts and observations:

A simple tandem repeat in the genomes of MFO individuals (diploid, sexually propagated) was analyzed by PCR using a pair of specific oligonucleotide primers. Their binding sites flanked the simple tandem repeat. One individual, A, was identified that produced a single 100 bp band and another, B, that produced a single 121 bp band. These two individuals were mated. The F1 progeny individuals all had the same pattern of bands. When F1 individuals were mated with each other, the F2 progeny produced exhibited three patterns of bands (including the two parental patterns).

In another approach, RAPD, DNA from A and B was analyzed by PCR using a single arbitrary primary and electrophoresis of the products. The A individual produced 5 bands. The B individual produced 4 bands, all matching bands from the A individual. As for the simple tandem repeat, the band patterns of the F1 progeny were indistinguishable. In contrast to the simple tandem repeat, the F2 individuals only had two distinct patterns.

Assignment (6 points):
Describe the F1 pattern for the simple tandem repeat.
Describe the F1 pattern for the RAPD.
Describe the non-parental F2 pattern for the simple tandem repeat.

Assignment (7 points):
Based on the above results, discuss the difference in the genetic information obtained by simple tandem repeat and RAPD analysis.
C. Facts and observations:

The numbers of distinct genes have been predicted from nucleotide sequences for several multicellular eukaryotic organisms. For *Caenorhabditis elegans, Oryza sativa, Arabidopsis thaliana, Fugu rubripes, Mus musculus,* and *Homo sapiens* the number is between 20,000 and 40,000, a 2-fold range. However, the genome sizes of these organisms vary from $97 \times 10^6$ to $3.3 \times 10^9$, a more than 30-fold range.

Assignment (12 points):
Account for the wider range in genome sizes than in numbers of genes.

III. Experimentation (30 points)
A. You have studied several vectors for molecular cloning of DNA in *E. coli*. The properties of these vectors are such that any particular vector is best suited for a limited size range of inserts.

Assignment:
Construct a table showing in one column the name of a vector type and in the second an approximate size range of inserts that can best be cloned in the vector. List as many as you wish, but only the first four will be graded (12 points). You may wish to consult the second assignment before proceeding.
Assignment:
From your table, constructed above, choose a vector appropriate to the tasks listed below and briefly justify your choice (9 points)

1. Cloning of near full-length cDNAs from mRNA (assume the average length of natural polypeptides to be about 300 amino acid residues).

2. Cloning of a bacterial operon (typically consisting of 3 to 10 genes).

3. Testing whether two genetically mapped molecular markers are within 200 kbp of one another on the chromosome.

B. You are directing a genomic library construction project for MFO. Your first attempt did not provide good coverage of all DNA segments in the genome.

Assignment:
Identify two variables that could be changed to increase the probability of recovering a particular DNA segment (5 points).

Assignment:
For each variable you identify, should the quantity be increased or decreased (4 points)?