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:
      Genetic, molecular, and morphological maps are said to be co-linear, meaning that the order of equivalent loci is the same on all three maps.

   Assignment (10 points):
      Describe how the orders of loci on genetic maps are determined.

   Assignment (10 points):
      Describe one way in which the orders of loci on molecular maps has been (or can be) determined.

   Assignment (7 points):
      Describe the morphological features of chromosomes that allow one to describe locations on the morphological chromosome.

   Assignment (8 points):
How can a molecular marker be assigned to a position on the morphological chromosome?

II. Interpretation (35 points total)
A. Facts and observations:

Baker’s/Brewer’s yeast (Saccharomyces cerevisiae) is a major model eukaryote for molecular genetic studies. It divides by budding, producing a small daughter cell and a large mother cell. This yeast can synthesize all amino acids needed for growth and can grow in a simple defined minimal medium.

Strain GWB lacks a gene, LEU2, for an enzyme in the biosynthetic pathway for the amino acid leucine. It can grow in minimal medium supplemented with leucine but not without the supplement.

The complete yeast LEU2 gene is available in a pBluescript-derived E. coli plasmid called pLEU. Methods for making yeast cells competent to take up DNA from their environment have been developed.

When competent yeast cells are mixed with pLEU linearized by treatment with HindIII and then plated on minimal medium agar (no leucine), a few colonies develop. DNA was extracted from each of these colonies and digested with Clal, an enzyme that has no sites for cleavage in pLEU. The digestion products were separated by gel electrophoresis and the fragments transferred to nylon membranes by Southern blotting. The blots were probed with labeled LEU2 DNA. Each sample showed a single band, but the bands varied in position in the gel, almost all having moved less far from the origin than HindIII-linearized pLEU.

Assignment (10 points):
Present a hypothesis for the location of the LEU2 gene in the transformed cells. The hypothesis should account for (please explain how it does!): the variability in size of hybridizing bands and the fact that they seem larger than pLEU, as well as the ability to grow in the absence of leucine.
B. Facts and observations:

When the above experiment was repeated substituting intact circular pLEU for the linearized pLEU DNA, no colonies were found on the agar.

*Hind*III linearized pLEU was mixed with total yeast DNA restricted with *Hind*III and the mixture treated with T4 DNA ligase and ATP. The ligation products (circular molecules) were mixed with competent GWB cells and plated on minimal medium agar (no leucine supplement). Several colonies resulted.

The total DNA from the colonies was analyzed by restriction, electrophoresis, Southern transfer and hybridization as above except that two separate digests were done, one with *Hind*III and the other with an enzyme that cleaved pLEU once at a position close to the *Hind*III site used for cloning. All colonies produced a single reactive *Hind*III fragment of the same mobility as the linearized pLEU. Single reactive bands were also produced when the other restriction enzyme was used, but the mobilities in electrophoresis were less than that of linearized pLEU.

Assignment (8 points):

Draw a map of the proposed structure of the *LEU2* containing DNA in the selected transformants. Be sure to label all the relevant features clearly.

Assignment (7 points):

What function did the yeast DNA ligated into pLEU likely provide that pLEU did not already have?
C. Facts and observations:

Some of the transformants from part B were grown for several passages separately in liquid culture (minimal medium) either supplemented or not supplemented with leucine. The cells were then plated on minimal medium agar lacking leucine. Those passaged without leucine present produced many colonies, while those passaged in the presence of leucine produced almost none.

Assignment (10 points):
Account for the difference in plating efficiency.

III. Experimentation (30 points)
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 five will be graded (15 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 (15 points)

1. Subcloning DNA fragments for Sanger dideoxy sequencing.

2. Cloning of full-length cDNAs from mRNA for the purpose of polypeptide expression (assume the average length of natural polypeptides to be about 300 amino acid residues).

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

4. Cloning of fragments of a eukaryotic genome to construct a scaffold for genome sequencing.

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