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

I. Interpretation (35 points total)

A. Facts and observations:
   1. The *Saccharomyces cerevisiae* RAD1 gene encodes a protein needed for one kind of repair of DNA damage, called nucleotide excision repair (NER). Cells defective in NER are more sensitive to killing by UV radiation. The Rad1 protein sequence is similar to the sequences of rad16 protein of *Schizosaccharomyces pombe* and MEI-9 of *Drosophila melanogaster*. These sequence similarities were used to isolate a plasmid clone containing a human cDNA insert that encodes a protein of similar amino acid sequence (Sijbers et al., 1996, Cell 86:811). The vector was an expression vector for eukaryotic cells.

   2. The human cDNA was used as a probe in fluorescent in situ hybridization (FISH) to human metaphase chromosome spreads. The authors concluded that the gene for the cDNA was located on the metacentric chromosome 16 in the interval 16p13.1-13.2.

Assignment:

Describe what the authors should have seen with the fluorescent microscope to allow them to make this assignment. (10 points)
3. Some rodent cell lines are defective in NER. One of these cell lines was used by others (Liu et al., 1993, Mutagenesis 8:199) to map a human gene involved in NER. Somatic hybridization was used in this mapping. The authors concluded that a complementing human gene is located in the 16p13.1-13.2 interval.

Assignment:
Describe what probably was done and what probably was observed in the somatic hybridization studies that resulted in this mapping (10 points)

4. Extracts of wild type rodent cell lines can catalyze all the reactions of NER. Two NER mutant cell lines whose extracts, when mixed, do not catalyze NER reactions are said to have mutations belonging to the same complementation group. Conversely, mutations of two NER mutant cell lines whose mixed extracts catalyze NER belong to different complementation groups.

5. The gene mutated in NER-defective rodent cell lines of complementation group 4 is called ERCC4. Cationic liposomes were used to transf ect rodent complementation group 4 cells with the human cDNA expression plasmid. Stable lines were selected. These resisted UV radiation as well as did wild type cells. The resistance was much better than for the untransfected cells.

6. Various forms of the human genetic defect xeroderma pigmentosum (XPA, XPB,...) are known. XP individuals have defects in DNA repair. Oligonucleotide primers were designed from the human cDNA clone nucleotide sequence. The primers were used to amplify cDNA sequences by reverse transcription-PCR from cells of XPF individuals. The nucleotide
sequences of these PCR products contained mutations relative to the cloned sequence.

7. The human cDNA expression clone was microinjected into fibroblast nuclei of an XPF individual. The resulting cells were more UV resistant than untreated XPF fibroblasts.

Assignment:
What is the probable relationship among ERCC4, XPF and Rad1? (5 points)

8. Gene X is known by FISH to be located closer to the centromere on the p arm of chromosome 16 than 16p13.1-13.2. Gene Y has a more telomeric location, also determined by FISH.

Assignment:
Predict the order of the three genes on the molecular map of chromosome 16. Which gene is closest to an end of the molecular map? (10 points)

II. Basis for principles (30 points)

A. Principle:
Highly repeated DNA in eukaryotic genomes occurs both in tandemly repeated blocks and interspersed with unique sequence DNA.

Assignment:
Give examples that support the above assertion OR describe experimental evidence that supports the assertion. (10 points)
B. Principle:
Template DNA strand separation and daughter DNA strand synthesis are closely coupled (occur obligatorily at the same time) during DNA replication in most organisms.

Assignment:
Describe one experimental observation supporting the above conclusion. (10 points)

C. Principle:
The separation according to size of populations of linear nucleic acid molecules with one end in common to all members of the population and the other end specific to a particular treatment is a common principle underlying several techniques used in molecular genetics.

Assignment:
Identify two techniques that use this principle. (10 points)

III. Experimentation (35 points)

Facts and observations:
1. The oxygen carrying protein of blood, hemoglobin, consists of heme and α- and β-globin polypeptide chains. The β-globin chain is encoded by a single gene at a known chromosomal location. Many RFLP and RAPD markers have been mapped to the region surrounding the β-globin locus. These markers have been tested and found to be stable (not undergoing mutation) throughout all multigenerational families examined.

2. A genetic defect has been described in humans that causes the production of a longer than normal β-globin chain. Precisely the same
genetic defect, the insertion of a single nucleotide within the \(\beta\)-globin coding region, is found in a number of families in several locations around the world.

3. That the same defect is found in a number of families could be a result of the same mutation occurring in different lineages. Alternatively, the families with the defective gene could have descended from one individual who lived in the distant past and in whom the mutation arose.

4. You have available DNA samples from two generations from families with the defect. These families come from several locations all over the world. You have available hybridization probes, restriction enzymes and PCR primers that can be used to assay the RFLP and RAPD markers linked to the \(\beta\)-globin locus. You also have all the typical equipment in a modern molecular genetics laboratory.

Assignment:

How do RAPD markers differ from RFLP markers in terms of the information they provide? (9 points)

Describe a strategy to distinguish between the mutation having occurred multiple times and the mutation having occurred just once. (12 points)
Describe the result expected from application of your strategy if the mutation occurred multiple times. (7 points)

Describe the result expected from application of your strategy if the mutation occurred just once. (7 points)