I. Interpretation (35 points total)

A. Facts and observations:
Bloom's syndrome is a rare genetic disorder characterized by small size, sun-sensitive facial erythema and immunodeficiency. Somatic cells of individuals homozygous for the Bloom's syndrome mutation accumulate excessive numbers of mutations. The mutation has been mapped to a limited area of the q arm of chromosome 15.

Most lymphoblastoid cell lines developed from such patients have an abnormally great number of sister chromatid exchanges (SCE's). A few lymphoblastoid cell lines developed from the same individuals have low (approximately normal) rates of SCE formation. In the low SCE lines, probing a variety of VNTR loci revealed that in the distal part of the p arm of chromosome 15, only one allele (that of one of the two parents) could be detected at each locus. Two alleles were present at loci in other parts of chromosome 15 and in other chromosomes.

Assignment: How could:
1) the low SCE lines have become homozygous for part of chromosome 15p; and
2) the lines have obtained the low SCE (normal) phenotype? (10 points)
One Bloom's syndrome patient was missing a VNTR marker locus known to be tightly linked to the Bloom's syndrome disease locus. A cosmid DNA containing the VNTR marker locus was used as a probe to isolate a clone from a commercial cDNA library. The nucleotide sequence of the clone was determined. A polypeptide of 1,417 residues was predicted from the open reading frame present.

The sequences of cDNA's from 13 patients were also analyzed. Each had a mutation. There were 7 different mutations. Three were missense substitutions. Four were predicted to result in premature polypeptide chain termination. These included:

a. A 1 bp insertion
b. A 6 bp deletion next to a 7 bp insertion
c. A 3 bp deletion
d. A one bp substitution.

Assignment: Explain how each of the four changes listed above could lead to premature polypeptide chain termination (5 points).

The polypeptide's sequence suggests that it is a DNA helicase.

Assignment: What processes might be affected by a defect in a DNA helicase? (5 points)
B. Facts and observations:

Development of many immunocompetent cells requires the activity of RAG-1 and RAG-2 (recombination-active) genes. In vitro produced derivatives of the encoded RAG-1 and RAG-2 proteins have recently been purified and analyzed for their activity on a synthetic DNA substrate:

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  .  16   |  7-12-9   |  6
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The double-stranded DNA contains a central heptamer-12 bp-nonamer sequence flanked by 16 bp and 6 bp of other sequence. The 5' end of the 16 bp flanking sequence is radioactively labeled.

Early during incubation of the DNA with a mixture of RAG-1 and RAG-2 proteins, a labeled 16 nucleotide fragment was detected by denaturing gel electrophoresis and autoradiography. Later a 32 nucleotide fragment was detected. This fragment was a hairpin (5' end complementary to the 3' end).

**Assignment:**

Identify and describe the process that these reactions are part of. (5 points)

What reactions are catalyzed by the complex of RAG-1 and RAG-2? (10 points)
II. Basis for principles (30 points)

A. Principle:
   During maturation of nuclear RNA transcripts to cytoplasmic messenger RNA, internal sequences are often removed.

Assignment:
   Describe one set of experimental observations that support the above statement. (5 points)

B. Principle:
   Molecular mechanisms for stabilizing chromosome ends have been conserved in evolution.

Assignment:
   Describe an experimental observation that supports the above statement. (5 points)

C. Principle:
   Molecular mechanisms for attachment of a chromosome's DNA to the mitotic spindle have been conserved in evolution.

Assignment:
   Describe an experimental observation that supports the above statement. (5 points)
D. Principle:
ARS (autonomously replicating sequences) are necessary but not sufficient for function as a bi-directional origin of replication

Assignment:
Describe an experimental observation that supports the above statement. (5 points)

E. Principle:
Recombination events are not uniformly distributed along the length of chromosomes. (There are hot spots for recombination).

Assignment:
Describe an experimental observation that supports the above statement. (5 points)

F. Principle:
The composition of a genome can change in response to environmental factors.

Assignment:
Describe an experimental observation that supports the above statement. (5 points)
III. Experimentation (35 points)

A. Facts and observations:
   1. Initiation of transcription of an important gene is known to be stimulated by binding of a protein factor.
   2. The binding is known to occur in a 200 bp region upstream of the transcription start site.
   3. Both the gene and the protein factor are available in purified form. In addition, you have available most common tools available in a molecular genetics lab, including (but not limited to): a vector and host cell system for introducing the gene, gel electrophoresis equipment, radioisotope license, reporter genes, X-Ray film development capability, centrifuges.

Assignment:
   Describe one way to more precisely define the binding site of the protein factor on the DNA. (15 points)

Describe the expected results of your test and how you would interpret them. (5 points)
B. Facts and observations:

1. The A1 protein of heterogeneous nuclear ribonucleoprotein particles (hnRNP A1) is bound to the poly A tails of each nuclear RNA and mRNA. It shuttles in and out of the nucleus so that in 6 h, half of the hnRNP A1 has moved out of the cytoplasm (replaced by new hnRNP A1 from the nucleus).

2. The amino acid sequence of hnRNP A1 does not contain a typical nuclear localization signal.

3. cDNA cloned in an expression vector is available, as is an antiserum against hnRNP A1. In addition, you have available most common tools available in a molecular genetics lab, including (but not limited to): a vector and host cell system for introducing the gene, gel electrophoresis equipment, radioisotope license, reporter genes and detection methods, X-Ray film development capability, centrifuges, immunocytochemistry tools.

Assignment:

Describe one way to identify the signal needed for transport of the protein into the nucleus. (10 points)

Describe the expected results of your test and how you would interpret them. (5 points)