DNA Technology
Chapter 20

1. Describe genetic engineering and DNA sequencing. (Genetic engineering is the direct manipulation of genes to achieve some practical goal. DNA sequencing is the process of determining the complete nucleotide sequence of a DNA molecule)

2. Describe how bacterial plasmids can be used to clone genes. (The piece of DNA to be cloned can be inserted into a plasmid obtained from a bacterial cell. The resulting plasmid (which is now a recombinant DNA molecule) is then returned to a bacterial cell. This single cell divides many times to form a population of genetically identical cells. Because the dividing bacteria replicate the recombinant plasmid and pass it on to their descendants, the foreign DNA and any genes it carries are cloned at the same time.)

3. Identify some reasons a researcher might clone a gene. (Producing proteins, producing genes for genetically modifying other cells, producing copies of the gene or the protein product for study.)

4. Describe the use of a restriction enzyme to create a recombinant DNA plasmid. (The same restriction enzyme is used to cut a bacterial plasmid and the DNA to be inserted into the plasmid (the gene of interest). Using the same restriction enzyme for both pieces of DNA produces sticky ends that will be complementary. The pieces of DNA are allowed to join, producing the recombinant plasmid. Note that the fragments of DNA will join in many different combinations, including the desired combination of the gene of interest in the bacterial plasmid.)

5. a) Identify the characteristic of DNA that causes it to move during gel electrophoresis. (DNA is negatively charged.)
   b) Identify the basis on which gel electrophoresis separates fragments of DNA. (Gel electrophoresis separates fragments of DNA based on their length.)

6. Describe how PCR can be used to produce many copies of a desired gene. (PCR requires double-stranded DNA containing the target sequence, a heat-resistant DNA polymerase, all four nucleotides, and two 15- to 20-nucleotide single DNA strands that serve as primers. One primer is complementary to one end of the target sequence on one strand; the second primer is complementary to the other end of the sequence on the other strand. The strands are separated so that the primers can attach to the complementary sequence at the end of each strand. DNA polymerase then copies both strands of the target sequence beginning at the primers. The process is repeated. Each repetition doubles the amount of DNA.)

7. If a researcher has genomic DNA, explain how a single, specific gene can be amplified using PCR. (By choosing primers that are complementary to the ends of the gene sequence, only that gene will be amplified.)

8. a) We know that DNA polymerase has an inherent error rate. Considering this, propose a reason that PCR cannot be used to produce an unlimited number of copies of a DNA molecule. (The occasional errors made by DNA polymerase would eventually result in

fragments of DNA that differ in sequence to the original sequence.)
b) Explain how living cells can be used to circumvent the limitations of PCR. (PCR can be used to provide DNA fragment for cloning. PCR primers are synthesized to include a restriction site at each end of the DNA fragment to be amplified. The resulting copies can then be inserted into a plasmid and cloned in living cells (which are able to correct the errors made by DNA polymerase.)

9. a) Describe the problem posed by introns in using bacteria to clone eukaryotic genes. (Bacteria lack the ability to process transcripts into mRNA so the introns cannot be removed. The intron sequences would be translated, producing long polypeptides.)
b) Explain how yeast can be used to avoid the incompatibility of eukaryotic genes in cloning using bacteria. (Yeast are eukaryotic cells, so are able to process introns. They are small and easy to grow so are good candidates for use in cloning genes.)

10. When using PCR to amplify a sample of DNA, the primers used are made of DNA, rather than RNA as in living cells. Suggest a reason for this. (The RNA primer must be replaced by DNA nucleotides, but DNA polymerase is incapable of starting from scratch at the 5' end of a new DNA strand. This results in a shorter daughter molecule with each replication (remember telomeres?). During PCR, using primers made of DNA, means they don't need to be replaced.)

11. a) When trying to determine which embryonic cells express a particular gene, researchers look for the corresponding mRNA in the cell. Propose a reason they would do so. (For a cell to express a gene, the gene would have first be transcribed into mRNA. The presence of a particular mRNA in a cell indicates the cell is expressing the associated gene.)
b) Describe how a nucleic acid probe can be used to detect the presence of a particular mRNA in a cell. (Using the cloned gene as a template, a short, single-stranded nucleic acid molecule complementary to the mRNA is synthesized. This molecule is called a probe. The probe is attached to (called labelling) a fluorescent molecule so that it can be easily detected. The probe is then added to a solution of the cells. If the mRNA is present, the probe will hybridize with it.)
c) Describe how you could determine which genes are expressed in a particular embryonic tissue compared to the same tissue in the fetus and the adult. (You would obtain tissue samples from the embryo, feus and adult and use a nucleic acid probe to determine if cells in each tissue contain the mRNA for the gene.)

12. Describe how the CRISPR-Cas9 system can be used to edit a gene. (Cas9 is a nuclease (like a restriction enzyme) that cuts double-stranded DNA molecules. Unlike restriction enzymes, the Cas9 protein will cut any sequence to which it is directed using a guide RNA molecule that it binds. The RNA molecule directs Cas9 to any complementary DNA sequence which it then cuts. A specific gene can be edited by introducing into cells a combination of Cas9 and an RNA complementary to the gene. The cutting of the DNA triggers a DNA repair system. Researchers include a segment from the normal (functional) gene along with the CRISPR-Cas9 system, and the DNA repair system uses this segment as a template to repair the cut DNA.)

13. In a DNA microarray, cDNA from normal tissue is labeled with a green fluorescent molecule

while cDNA from cancerous tissue is labeled with a red fluorescent molecule. State which color spots would represent genes you would be interested in if you were studying cancer. Justify your response. (You would be interested in both green and red spots because these are the genes for which the expression might differ between the two tissues. Some of them might cause or prevent cancer.)