You must know:

- The terminology of biotechnology
- The steps in gene cloning with special attention to the biotechnology tools that make cloning possible.
- The key ideas that make PCR possible.
- How gel electrophoresis can be used to separate DNA fragments or protein molecules.
1. Bacteria can transfer DNA via conjugation, transformation, and transduction. Match the following statements with one of the methods of bacterial DNA transfer. (Some statements are true of all methods of DNA transfer.)

A. Conjugation  ____ 1  What happened in Griffith's experiment with pneumonia bacteria
B. Transformation  ____ 2  DNA may be integrated into chromosome of recipient
C. Transduction  ____ 3  Taking up of DNA from the fluid surrounding a cell
D. All three of the above  ____ 4  Alters genetic makeup of recipient cell

____ 5  Figure 5 below
____ 6  Male and female cells joined by sex pili
____ 7  Figure 7 below
____ 8  Bacterial "mating"
____ 9  Figure 9 below
____ 10  Creates a recombinant cell
____ 11  Transfer of genes by a bacteriophage
____ 12  May involve transfer of genes by a plasmid
____ 13  Usually controlled by a piece of DNA called an F factor

2. Plasmids can be used to engineer bacteria to produce desired genes or proteins. Review techniques used to splice and clone genes by filling in the blanks.

Gene engineers use plasmids as 1 ____________ to insert genes into bacteria or eukaryotic cells. Imagine that you wanted to build a bacterium capable of making large quantities of human growth hormone (HGH), which is a protein. Your first step would be to obtain the 2 ____________ that codes for HGH. One way to do this is to use a 3 ____________ enzyme to cut up all the DNA in a human cell. The enzyme recognizes short nucleotide 4 ____________ within DNA molecules and cuts the DNA at specific points in these 5 ____________ sequences. Restriction enzymes cut the two DNA strands unevenly, leaving single-stranded ends that can hydrogen-bond with complementary single-stranded 6 "__________ ends." A restriction enzyme can chop up a cell's DNA into thousands of pieces, each consisting of a few genes.

The next step in making human growth hormone is isolating a supply of 7 ____________ to use as vectors, for carrying the DNA fragments into bacteria. These are treated with the same restriction enzyme that was used to cut up the human DNA, producing plasmids with sticky ends that are 8 ____________ to sticky ends of the human DNA fragments.

Now the human DNA fragments are mixed with plasmids. The sticky ends on the fragments base-pair with the sticky ends on the plasmids, but these connections are weak and temporary. An enzyme called DNA 9 ____________, which normally functions in DNA 10 ____________ is used to catalyze the formation of covalent bonds between adjacent nucleotides in the DNA fragments and plasmids. This forms 11 ____________ DNA, a DNA molecule with a new, human-made combination of genes.

In the next step, each recombinant plasmid is added to a bacterium. Under specific conditions, a bacterium will take up the plasmid DNA from solution by the process of 12 ____________. The bacterium, with its recombinant plasmid, is allowed to grow
and reproduce on a nutrient medium. Each bacterium replicates its own DNA and the plasmid DNA and then divides repeatedly. Each bacterium grows into a colony of identical cells, all containing the recombinant DNA. This production of multiple copies of the genes is called gene cloning. Cloning all the different DNA fragments obtained from the human cell produces a genomic library of DNA segments. Because this procedure does not target a particular gene (at least so far), it is called the "shotgun approach" to gene cloning. (DNA fragments can also be spliced into phages, that infect bacteria. The phages reproduce in bacteria to produce libraries of cloned DNA pieces.)

There are a lot of genes to sort through in a library produced from an entire eukaryotic genome. Plus, eukaryotic genes contain noncoding sequences, which must be removed before bacteria can read them. It is often better to start with the genes expressed in a particular kind of cell, using the enzyme transcriptase to produce intron-free genes. If you wanted to obtain a human growth hormone gene, the place to start would be a cell from the pituitary gland, where HGH is made. In the cell, the HGH gene (and others) is transcribed into RNA. Enzymes then remove the introns from the RNA and splice the remaining together to make mRNA. The mRNA is then extracted from the cell, and reverse transcriptase (obtained from a ) is added. The reverse transcriptase transcribes a strand of DNA along the mRNA molecule. The RNA is then broken down, and a second DNA strand is synthesized, producing double-stranded DNA. This artificial gene lacks introns, so it is more manageable than the original gene. It also can be transcribed and translated by , which lack the ability to splice RNA. Artificial genes produced in this fashion are cut and pasted into plasmids, using restriction enzymes and ligase, and then cloned in bacteria. There are many mRNA molecules in a pituitary gland cell, so this method also produces a library; however, this library is smaller than a library produced by cutting up the entire genome, because it is limited to the genes actually in a pituitary gland cell.

At this point you have isolated and cloned the HGH gene, but where is it? A genomic library can consist of thousands of bacterial colonies. The bacteria of one of the colonies contains the HGH gene, but which one? One way to look for the gene is to look for HGH, its product. But usually you look for the gene itself, a search that is made easier by using a nucleic acid isotope and is called a probe because it can be used to find the gene. To find the bacterial clone that holds the gene, DNA is obtained from each colony of bacteria and treated to separate the DNA strands. The probe is then mixed with the DNA strands, and it hydrogen-bonds only with the recombinant DNA with a complementary base sequence - the HGH gene. Once you have figured out which bacterial colony in the library contains the HGH gene, you can grow these bacteria in larger amounts.

If you know the nucleotide sequence of the desired gene, you can bypass much of the trouble of cloning and searching for the gene by simply synthesizing it in the laboratory. There are now gene machines that can put together artificial genes several hundred in length. There are also automatic DNA sequencers capable of determining the sequences of large genes in a day or so. The genes are cut into fragments by restriction enzymes and machine-analyzed. The sequences are then fed into computer data banks for interpretation.

The final step in engineering bacteria to produce human growth hormone is to grow the bacteria in large quantities (usually done in large vats) and extract the protein. The bacteria will manufacture the protein on command if you have spliced the proper control sequences into your recombinant plasmids. Now it is only necessary to collect and purify the protein (and get approval from the Food and Drug Administration!) to start treating patients with recombinant DNA HGH.
3. Review recombinant DNA techniques by matching each of the diagrams (or parts of diagrams) below with one of the following processes: isolating plasmid from E. coli; extracting DNA from a eukaryotic cell; obtaining copies of gene and protein from cloned bacteria; cutting DNA with restriction enzyme; joining plasmid and DNA fragment using DNA ligase; cloning recombinant DNA; using reverse transcriptase to make an artificial gene; using a nucleic-acid probe to find a gene; inserting a plasmid into a bacterium via transformation; and mixing plasmids and DNA fragments with sticky ends.
4. Powerful molecular biology techniques now allow us to amplify, analyze, and compare genes. Review these methods by matching each phrase on the right with a term on the left.

A. RFLP analysis  _____  1  Transferring DNA to paper: named for its discoverer
B. carrier  _____  2  Used to cut up DNA for analysis
C. gel  _____  3  Piece of DNA cut up by restriction enzymes
D. recognition sequence  _____  4  Place where enzyme cleaves DNA
E. positive pole  _____  5  Restriction fragment length polymorphism
F. restriction fragment  _____  6  Type of cell often used in RFLP analysis
G. DNA polymerase  _____  7  Separates DNA fragments by size and electrical charge
H. RFLP  _____  8  Restriction fragments move through this
I. Southern blotting  _____  9  Restriction fragments are attracted to this
J. white blood cell  _____  10  Where specific restriction fragment collects in gel
K. band  _____  11  Chromosomal "landmark" that can be studied
L. restriction enzyme  _____  12  Comparing restriction fragment patterns
M. genetic marker  _____  13  Method for making many copies of a DNA molecule
N. gel electrophoresis  _____  14  Used to replicate DNA in a test tube for PCR method
O. polymerase chain reaction (PCR)  _____  15  Heterozygote who might possess a harmful allele

5. Pages 242-243 give a number of facts and figures that will help put the human genome into perspective. It is not important to memorize the figures, but it is important for you to get an idea of how big and how small some of these things are. Choose the correct number to complete each of the statements. Choose from 10, 23, 46, 97, 1000, 2000, 100,000, 130 million, and 3 billion.

1. Number of chromosomes in a diploid human cell: _______________
2. Number of chromosomes in a haploid set: _______________
3. Number of nucleotide pairs in a haploid set of human chromosomes: _______________
4. Number of nucleotide pairs in an average human chromosome: _______________
5. Estimated number of genes in a human cell: _______________
6. Number of genes in E. coli: _______________
7. Amount of DNA in a human cell divided by amount of DNA in E. coli: _______________
8. Percentage of DNA in a human cell that is thought to be noncoding DNA: _______________
9. Amount of human DNA in introns divided by amount in exons: _______________
6. This schematic diagram shows the steps in plasmid cloning of a gene. Identify components a-j.

7. A bloody crime has occurred. Police have collected blood samples from the victim, two suspects, and blood found at the scene. Briefly list the steps the lab went through to produce the following autoradiograph.

a. 

b. 

c. 

d. 

e. 

Which suspect would you charge with the crime? Explain.
8. This segment of DNA has restriction sites I and II which create restriction fragments a, b, and c. Which of the following gel(s) produced by electrophoresis would represent the separation and identity of these fragments?

![Diagram of gel patterns](image)

9. This restriction fragment contains a gene whose recessive allele is lethal. The normal allele has restriction sites for the restriction enzyme PSTI at sites I and II. The recessive allele lacks restriction site I. An individual who had a sister with the lethal trait is being tested to determine if he is a carrier of that allele. Indicate which of these bands patterns would be produced on a gel if he is a carrier (heterozygous for the gene)?

![Diagram of gel patterns](image)