North Salem University

<u>MISSION</u>: Engage students to continuously learn, question, define and solve problems through critical and creative thinking.

Interactive Student Study Guide Spri2024

AP

Biology

Chapter 20: DNA Technology and Genomics

Chapter 21: The Genetic Basis of Development

The AP Biology exam has reached into these chapters for FRQ questions on a regular basis. Student responses show that biotechnology is a difficult topic. This chapter requires a strong conceptual understanding of the technological processes and the underlying biochemistry of DNA that guides the procedure. With a little careful work, this chapter will give you insights into the incredible advancements already made and a basis for understanding the new marvels yet to be discovered in the fields of molecular genetics and biotechnology.

If you have any problems – please sign up for extra help after school.

Chapter 20: DNA Technology and Genomics

OBJECTIVES

DNA Cloning

- __1. Explain how advances in recombinant DNA technology have helped scientists study the eukaryotic genome.
- ____2. Describe the natural function of restriction enzymes.
- __3. Explain how the creation of sticky ends by restriction enzymes is useful in producing a recombinant DNA molecule.
- ___4. Outline the procedures for cloning a eukaryotic gene in a bacterial plasmid.
- ___5. Describe the role of an expression vector.
- ____6. Describe two techniques to introduce recombinant DNA into eukaryotic cells.
- ____7. Define and distinguish between genomic libraries using plasmids, phages, and cDNA.
- __8. Describe the polymerase chain reaction (PCR) and explain the advantages and limitations of this procedure.

DNA Analysis and Genomics

- ___9. Explain how gel electrophoresis is used to analyze nucleic acids and proteins and to distinguish between two alleles of a gene.
- __10. Describe the process of nucleic acid hybridization.
- ___11. Describe the Southern blotting procedure and explain how it can be used to detect and analyze instances of restriction fragment length polymorphism (RFLP).
- ___12. Explain how RFLP analysis facilitated the process of genomic mapping.
- __13. List the goals of the Human Genome Project.
- ___14. Explain how linkage mapping, physical mapping, and DNA sequencing each contributed to the genome mapping project.
- ___15. Describe the alternate approach to whole-genome sequencing pursued by J. Craig Venter and the Celera Genomics company. Describe the advantages and disadvantages of public and private efforts.
- __16. Describe the surprising results of the human genome project.
- ___17. Explain how the vertebrate genome, including that of humans, generates greater diversity than the genomes of invertebrate organisms.
- __18. Describe what we have learned by comparing the human genome to that of other organisms.
- __19. Explain how in vitro mutagenesis and RNA interference help to discover the functions of some genes.
- ____20. Define and compare the fields of proteomics and genomics.
- ___21. Explain the significance of single nucleotide polymorphisms in the study of the human genome.

Practical Applications of DNA Technology

- ___22. Describe how DNA technology can have medical applications in such areas as the diagnosis of genetic disease, the development of gene therapy, vaccine production, and the development of pharmaceutical products.
- ___23. Explain how DNA technology is used in the forensic sciences.
- ___24. Describe how gene manipulation has practical applications for environmental and agricultural work.
- _25 Explain how DNA technology can be used to improve the nutritional value of crops and to develop plants that can produce pharmaceutical products.
- ___26. Describe the safety and ethical questions related to recombinant DNA studies and the biotechnology industry
- ___27. Describe the general process by which the ewe Dolly and the first mice were cloned.
- ____28. Describe the two important properties of stem cells. Explain their significance to medicine.

KEY TERMS:

biotechnology cDNA library complementary DNA (cDNA) DNA fingerprint DNA ligase gel electrophoresis genetically modified organisms (GMOs) genetic engineering Human Genome Project recombinant DNA restriction enzyme restriction fragment length polymorphisms (RFLPs) stem cells (embryonic/adult) sticky end chromosome walking cloning vector DNA microarray assays expression vector gene cloning gene therapy genomic library genomics polymerase chain reaction (PCR) restriction site single nucleotide polymorphisms (SNPs) stem cells (totipotent/pluripotent)

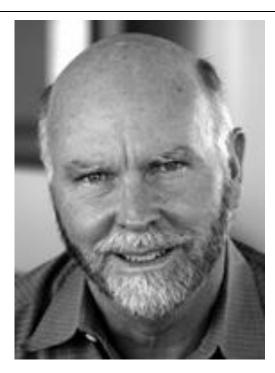
WORD ROOTS:

liga- = bound, tied (DNA ligase: a linking enzyme essential for DNA replication)

electro- = electricity (electroporation: a technique to introduce recombinant DNA into cells by applying a brief electrical pulse to a solution containing cells)

muta- = change; -genesis = origin, birth (in vitro mutagenesis: a technique to discover the function of a gene by introducing specific changes into the sequence of a cloned gene, reinserting the mutated gene into a cell, and studying the phenotype of the mutant)

poly- = many; morph- = form (Single nucleotide polymorphisms: one-base-pair variations in the genome sequence)



John Craig Venter

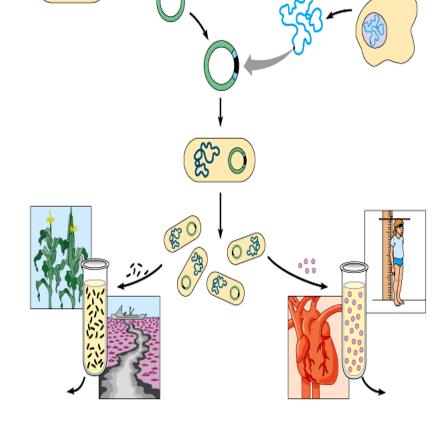
Guided Reading: Chapter 20

- 1. It is important to understand the meaning of the three terms in below to start this chapter.
 - (a) recombinant DNA –
 - (b) genetic engineering –
 - (c) biotechnology -
- 2. *Plasmids* are important in biotechnology. Give a full and complete definition of *plasmid*.
- 3. The production of multiple copies of a single gene is called ______
- 4. Using Figure 20.1, label and explain the five steps in this preview of *gene cloning*.
- (1)
- (2)

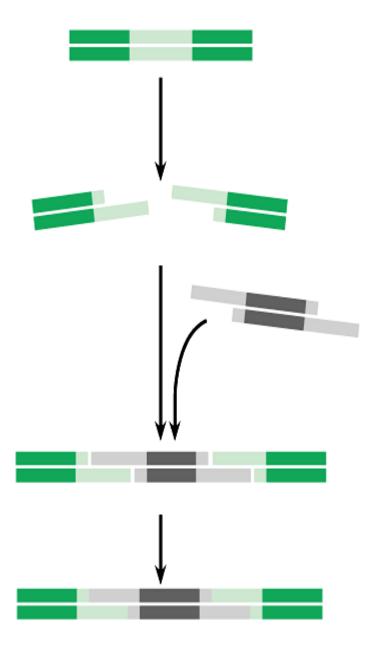
(3)

(4)

(5)



5. Read the description of *restriction enzymes* on page 377 carefully. Then, using Figure 20.2 as a guide, label and explain each step in the diagram below.



6. When were *restriction enzymes* discovered and what function do they serve in nature?

7. What is a *cloning vector*?

- 8. Figure 20.3 is a more detailed discussion of the gene cloning procedure shown in Figure 20.1. Explain the following key points.
 - (a) Explain why the plasmid is engineered with ampR gene.

(b) After transformation has occurred, why are some colonies blue?

(c) Why are some colonies white? Why is this important?

- 9. Describe how a radioactively labeled nucleic acid probe can locate the gene of interest. (Use Figure 20.4 to guide your response.)
- **10.** The *cloning* procedure described in question 8 and Figure 20.3 will produce many different fragments of DNA. These fragments may be stored in a genomic library. What is the purpose of a genomic library?

11. The *polymerase chain reaction* (*PCR*) is a Nobel Prize - winning idea that is used by scientists to amplify DNA, particularly when the quantity of DNA is very small or contaminated. Label the diagram to the right and use it to name and briefly explain the three initial steps that occur in PCR.

(1)

(2)

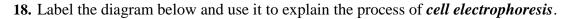
(3)

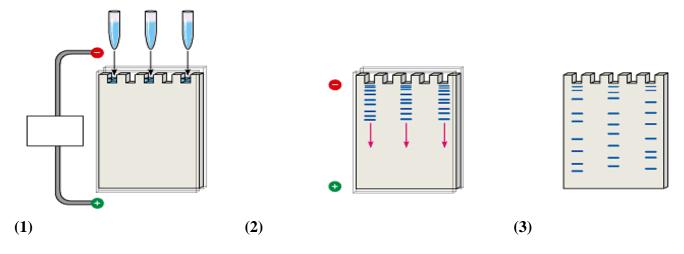


12. How many molecules will be produced by four PCR cycles?

The next section begins our study of *gel electrophoresis*, a technique that was covered in Honors Biology. (*It is important to understand the principles of gel electrophoresis.*)

- 13. ______ is a technique used to separate nucleic acids or proteins that differ in *size* or *electrical charge*.
- 14. Why is the DNA sample to be separated by *gel electrophoresis* always loaded at the cathode or negative end of the power source?
- 15. Why is DNA treated with *restriction enzymes* before analyzed via gel electrophoresis.
- 16. What are *restriction fragment length polymorphisms* (*RFLP's*)?
- 17. Explain why shorter DNA fragments or *RFLP*'s travel farther down the gel than larger molecules.





19. What is the *Human Genome Project*?

20. What was one surprising result of the Human Genome Project?

21. So what makes human - and vertebrate animals in general - more complex than flies or worms?

22. What is *proteomics*?

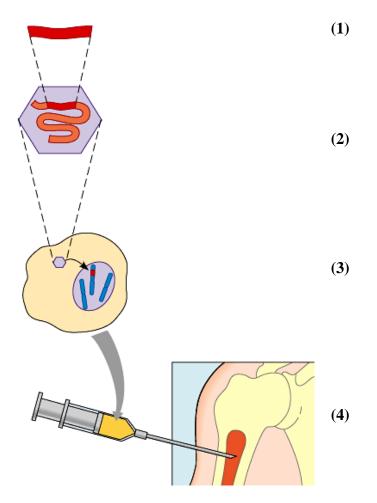
23. The amount of DNA variations in humans is small compared to other species. Why is this?

24. Most of the variations in our DNA seems to be in the form of *single nucleotide polymorphisms* (SNP's). What are *single nucleotide polymorphisms* (SNP's)?

25. How has DNA technology helped in the diagnosis and treatment of human genetic diseases?

26. What is gene therapy?

27. Label the diagram below and use it to describe one type gene therapy.



28. Why hasn't gene therapy proven to be very effective at correcting genetic defects in human somatic cells?

29. If not for correcting genetic defects, then what are researcher's currently using gene therapy for?

30. What was one of the first practical applications of gene splicing in the pharmaceutical industry?

31. How do Forensic Scientists use RFLP analysis by Southern blotting to solve murder cases?

32. Based upon the DNA fingerprint to the right, which suspect's DNA matches the DNA found at the crime scene? Explain your answer. (*Murder Mystery Review*)

Crime scene	1	Suspect 1	Suspect 2	Suspect 3
\equiv	1	_	\equiv	—
—	I	=	—	=
-	1		-	
—	1	—	—	-
_	1	_	_	-
	Ì			-

33. How is genetic engineering/DNA technology being applied to environmental work?

34. How is genetic engineering/DNA technology being used in agriculture, specifically animal husbandry?

35. How is genetic engineering/DNA technology being used in agriculture, specifically crop production?

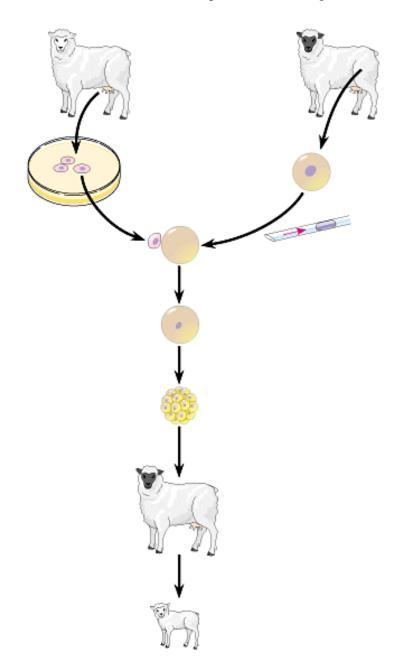
36. What are <u>**G**</u>enetically <u>**M**</u>odified <u>**O**</u>rganisms (GMO's)?

37. What are some of the major safety concerns relating to DNA technology?

38. What are some of the major ethical concerns relating to DNA technology?

Guided Reading: Chapter 21

39. Label the diagram below and use it to describe the process of cloning in mammals.



40. In most cases, only a tiny percentage of cloned embryos develop normally. Why is this?

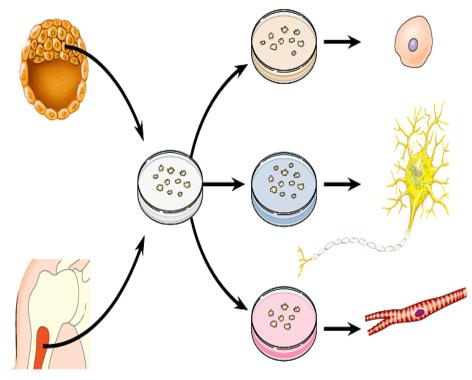
41. Embryonic development involves *cell division* (mitosis + cytokinesis), *cell differentiation* and *morphogenesis*. Define *cell differentiation* and *morphogenesis*.

42. What is a *totipotent* cell?

43. What are *stem cells* and what two important properties do they possess?

44. Label the diagram below and use it to describe the benefits of stem cell research.

Benefits:



45. What are *pluripotent* stem cells?

46. What is the major difference between *embryonic stem cells* and *adult stem cells*?

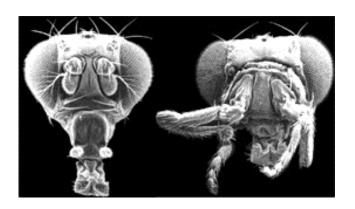
47. Why does *embryonic* stem cell research raise a number of ethical and political concerns?

48. What are **homeotic genes**?

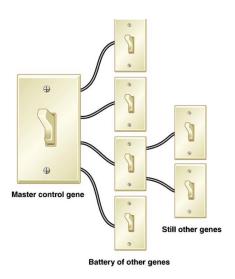
49. Homeotic genes produce transcription factors or regulatory proteins. What do these regulatory proteins control?

50. What is a **homeobox**?

- **51.** Homeobox-containing genes are often called ______, especially in mammals.
- **52.** Use the diagram to the right to help you explain the effects of mutations in homeotic genes.



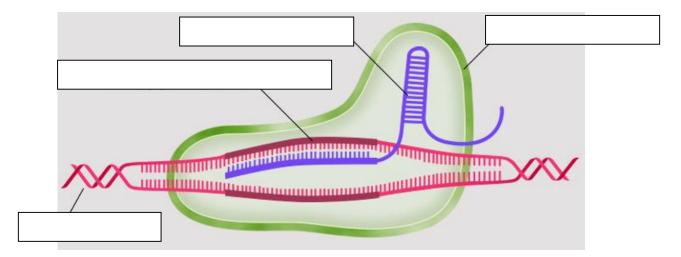
53. Use the diagram to the right to help you explain other functions of Hox genes.



Base your answer each of the following questions on the video CRISPR located on the AP Biotechnology page of Collea's Corner.

- 54. What does **CRISPR** stand for?
- 55. Name the 2 components that make up **CRISPR** and briefly describe their functions.

56. Label the **CRISPR** diagram below.



57. List and briefly describe the main steps in the **CRISPR** process.

58. What do scientist hope to use **CRISPR** for in the future?

Chapter 20: Summary of Key Concepts

DNA CLONING

- DNA technology makes it possible to clone genes for basic research and commercial applications: an overview (pp. 376-377, FIGURE 20.1) DNA technology is a powerful set of techniques that enables biologists to manipulate and analyze DNA. It can help make useful new products and organisms.
- Restriction enzymes are used to make recombinant DNA (pp. 377-378, FIGURE 20.2) A variety of bacterial restriction enzymes recognize short, specific nucleotide sequences in DNA and cut the sequences at specific points on both strands to yield a set of double-stranded DNA fragments with single-stranded sticky ends. The sticky ends readily form base pairs with complementary single-stranded segments on other DNA molecules. The enzyme DNA ligase can seal the strands to produce recombinant DNA molecules.
- Genes can be cloned in recombinant DNA vectors: a closer look (pp. 378-381, FIGURES 20.3-20.5) Plasmids can serve as vectors (carriers) to introduce foreign genes into host bacteria. Recombinant DNA is made by inserting restriction fragments from DNA containing a gene of interest into the vector DNA, which has been cut open by the same enzyme. Gene cloning results when the foreign genes replicate inside the host bacterial cell as part of the recombinant vector. Eukaryotic cells can also serve as host cells for gene cloning. Cell clones carrying the gene of interest can be identified with a radioactively labeled nucleic acid probe, which has a sequence complementary to the gene.
- Cloned genes are stored in DNA libraries (pp. 381-382, FIGURE 20.6) When the starting material for DNA (gene) cloning is an entire genome, the resulting collection of recombinant vector clones is called a genomic library. Alternatively, a cDNA (complementary DNA) library can be made by cloning DNA made in vitro by reverse transcription of all the mRNA produced by a particular kind of cell. Libraries of cDNA are especially useful for working with eukaryotic genes (whose introns are not present in the cDNA versions) and for studying gene expression.
- The polymerase chain reaction (PCR) clones DNA entirely in vitro (pp. 382-383, FIGURE 20.7) For quickly making many copies of a particular segment of DNA, this method uses primers that bracket the desired sequence and a heat-resistant DNA polymerase.

DNA ANALYSIS AND GENOMICS

- Restriction fragment analysis detects DNA differences that affect restriction sites (pp. 383-386, FIGURES 20.8-20.10) Gel electro-phoresis makes it possible to separate and isolate DNA restriction fragments of different lengths. Restriction fragment length polymorphisms (RFLPs) are differences in DNA sequence on homologous chromosomes that result in different patterns of restriction fragment lengths. These patterns are visualized as bands on gel electrophoresis. Specific fragments can be identified by Southern blotting, using labeled probes that hybridize to the DNA stuck to a "blot" of the gel. RFLPs are prevalent genetic markers, present throughout eukaryotic noncoding DNA. RFLP analysis has many applications, including genetic mapping and diagnosis of genetic disorders.
- Entire genomes can be mapped at the DNA level (pp. 386-389, FIGURES 20.11-20.13) An international research effort, the Human Genome Project involves linkage mapping, physical mapping, and DNA sequencing of the human genome and the genomes of other organisms. An alternative approach starts with sequencing of random DNA fragments, relying especially heavily on computer power to assemble the sequences. The human genome is thought to have 30,000 to 40,000 genes, fewer than once thought.
- Genome sequences provide clues to important biological questions (pp. 389-393, FIGURE 20.14) Genome sequences are helping researchers find new genes, probe details of gene organization and control, and answer questions about evolution. DNA microarrays allow researchers to compare patterns of gene expression in different tissues and under different conditions. Genomics is the systematic study of entire genomes; proteomics is the systematic study of all the proteins encoded by a genome. Single nucleotide polymorphisms (SNPs) provide useful markers for studying human genetic variation.

PRACTICAL APPLICATIONS OF DNA TECHNOLOGY

- DNA technology is reshaping medicine and the pharmaceutical industry (pp. 393-395; FIGURES 20.15, 20.16) Medical applications of DNA technology include diagnostic tests for genetic and other diseases; safer, more effective vaccines; the large-scale production of many new, and some previously scarce, pharmaceutical products; and the prospect of treating or even curing certain genetic disorders.
- DNA technology offers forensic, environmental, and agricultural applications (pp. 395-399; FIGURES 20.17-20.20) DNA "fingerprints" obtained from RFLP or STR analysis of tissue found at the scenes of violent crimes provide evidence in trials; such fingerprints are also useful in parenthood disputes. Genetic engineering can modify the metabolism of microorganisms so that they can be used to extract minerals from the environment or degrade waste materials. In agriculture, transgenic plants and animals are being designed to improve food productivity and quality.
- DNA technology raises important safety and ethical questions (p. 399) Several U.S. government agencies are responsible for setting policies about and regulating recombinant DNA technology. The potential benefits of genetic engineering must be carefully weighed against the potential hazards of creating products or developing procedures that are harmful to humans or the environment.

Chapter 21: Summary of Key Concepts

FROM SINGLE CELL TO MULTICELLULAR ORGANISM

- Embryonic development involves cell division, cell differentiation, and morphogenesis (p. 403) In addition to mitosis, embryonic cells undergo differentiation, becoming specialized in structure and function. Morphogenesis encompasses the processes that give shape to the organism and its various parts.
- Researchers study development in model organisms to identify general principles (pp. 403-406,FIGURE 21.3) Organisms used to study the genetic basis of development include the fruit fly Drosophila melanogaster, the nematode Caenorhabditis elegans, the mouse Mus musculus, the zebrafish Danio reri, and the common wall cress Arabidopsis thaliana.

DIFFERENTIAL GENE EXPRESSION

• Different types of cells in an organism have the same DNA (pp. 406-410;FIGURES 21.5,21.6) Cells differ in structure and function not because they contain different genes, but because they express different portions of a common genome; they have genomic equivalence. Differentiated cells from mature plants are often totipotent, capable of generating a complete new plant. The nucleus from a differentiated animal cell can sometimes give rise to a new animal if transplanted to an enucleated egg cell. Pluripotent stem cells from animal embryos or adult tissues can reproduce and differentiate in vitro as well as in vivo, offering hope for medical use.

Chapter 20/21 - Review Questions

_1)	Biotechnology -A) is a modern scientific discipline that has existed for only a few decades.B) is strictly concerned with the manipulation of DNA.C) has been around since the dawn of civilization.D) is generally considered more harmful than valuable to society.						
2)	When DNA from two sources is combined into one single piece of DNA, it is known as -						
	A) cloned DNA.B) recombinant DNA.	C) a vector. D) a plasmid.					
3)	The production of multiple identical copies of gene-sized pieces of DNA defines -						
	A) gene cloning.	C) clonal selection.					
	B) plasmid transformation.	D) tissue culturing.					
4)	In the process of human gene cloning using plasmids, the bacterial plasmid - A) functions as a vector.						
	B) is the source of the gene to be cloned.						
	C) is cultured inside the human cell, which contains the gene to be cloned.D) is used to insert the human gene into the bacterial chromosome.						
5)	DNA ligase binds -						
	A) exons together.	C) nucleotides together.					
	B) polymerase to the promotor.	D) an intron to an exon.					
6)	Restriction enzymes -						
	A) edit proteins.	C) stop transcription.					
	B) cut DNA at specific sites.	D) bind together strands of DNA.					
7)	Restriction enzymes specifically recognize and cut short sequences of DNA called -						
/	A) promoter sequences.	C) sticky ends.					
	B) short terminal repeats.	D) restriction sites.					
8)	"Sticky ends" are -						
()	A) produced by the action of DNA ligase.						
	B) produced by PCR.						
	C) always long sequences of a single nucleotide.						
	D) DNA fragments with exposed single-stranded ends.						
9)	The feature of "sticky ends" that makes them especially useful in DNA recombination is their ability to						
- /	A) bind to DNA and thereby activate transcription.						
	B) bind to ribosomes and thereby activate translation.						
	C form budge on bounded been noise with complementary	wain all atmosphere and atmosphere of DNA					

- C) form hydrogen-bonded base pairs with complementary single-stranded stretches of DNA.
- D) allow plasmids to attach to the main bacterial chromosome.

_10) A collection of DNA fragments obtained from the genome of one organism, inserted by recombinant DNA techniques into the genome of a host organism (one fragment per host genome), and maintained there is called a -

A) DNA collection.

B) genomic library.

C) DNA file.D) gene bank.

__11) The enzyme that converts information stored in their RNA to information stored in DNA is -

A) DNA ligase.B) reverse transcriptase.

C) a restriction enzyme. D) RNA polymerase.

- __12) A nucleic acid probe is -
 - A) a virus that transfers DNA to a recipient cell.
 - B) a piece of radioactively labeled DNA that is used to locate a specific gene.
 - C) an enzyme that locates a specific restriction site on RNA.
 - D) a plasmid that recognizes a specific DNA sequence.
- ____13) Which of the following statements about nucleic acid probes is *false*?
 - A) A nucleic acid probe is a double-stranded section of DNA.
 - B) A nucleic acid probe can be used to find a specific gene.
 - C) A nucleic acid probe binds to a complementary sequence in the gene of interest.

D) A nucleic acid probe is usually labeled with a radioactive isotope or fluorescent tag to help identify its location.

__14) The advantage of being able to clone the gene for human insulin is that -

- A) there are too few cows, pigs, and horses to provide an adequate supply of their insulin.
- B) human insulin is less likely to cause harmful side effects than cow, pig, or horse insulin.

C) cow, pig, or horse insulin cannot keep a diabetic alive for more than three months.

D) using human insulin increases the probability that, in the future, the person suffering from diabetes can be weaned from a dependence on insulin.

- ____15) Golden rice is golden in color because it is rich in -
 - A) vitamin A.B) vitamin C.
- C) beta-carotene. D) chromium picolinate.

__16) A transgenic animal is -

A) an animal that is the first of its kind to bear a particular allele.

- B) an animal in which a genetic defect has been corrected using recombinant DNA therapy.
- C) an animal containing a gene from another organism, typically of another species.
- D) an animal containing genes from three or more species.
- __17) In order for gene therapy to be permanent, -
 - A) the defective gene must first be removed from all somatic cells.
 - B) the normal gene must be added to the germ line cells.
 - C) the normal gene must first be treated with UV radiation to ensure noninfectivity.
 - D) the normal gene must be transferred to somatic cells that can continuously multiply.

other organic matter from you	f you commit a crime, you need to make sure that you do not leave even the smallest speck of blood, hair, or ther organic matter from your body. If you do, the DNA in this material can be amplified by, ubjected to genetic analysis, and used to identify you as the perpetrator of the crime. A) PCR					
B) blotting		D) reverse transcr	iptase			
19) The polymerase chain reaction living in hot springs.	-					
A) DNA polymerase molecule	es	C) restriction enzy	/mes			
B) phages		D) plasmids				
20) Gel electrophoresis sorts DNA						
A) nucleotide sequence.		C) solubility in the	e gel.			
B) ability to bind to mRNA.		D) size.				
21) During the process of electrop	bhoresis, the func	tions like a molecul	ar sieve, separating the samples			
according to their size.						
A) sample mixture	la	C) negatively char	rged electrode			
B) positively charged electrod	le	D) gel				
22) Segments of eukaryotic DNA	that can move or be copied	from one site to and	other in the genome are called -			
A) exons.	I		ements or transposons.			
B) plasmids.		D) vectors.				
 23) Which of the following statem A) Proteomics is the study of B) Proteomics involves the co C) Proteomics is the systematic D) Proteomics and genomics and genomics 	protein interaction within a omplete analysis of the prok ic study of the full set of pro	cell. aryotes. oteins encoded by a				
24) Approximately what percentage of the human genome is identical to that of a chimpanzee?						
A) 50.0%	B) 62.3%	C) 92.0%	D) 98.8%			
25) In recombinant methods, the t	erm "vector" refers to -					
A) the "sticky" ends of a DNA		C) an RFLP marke	er			
B) a DNA probe used to locat	÷	,	her agent used to transfer DNA			
26) A genetic marker is -						
A) a place where a restriction	•					
B) a chart that traces the famil						
· · · ·	C) a particular nucleotide sequence whose inheritance can be followed.					
D) a radioactive probe used to	iniu a gene.					
27) Putting a human gene into the	plasmids of bacteria has er	abled scientists to -				
A) insert the corrected gene into patients who have certain genetic disorders.B) use these bacteria to mass-produce mRNA for certain genes.						
D) use bacteria as "factories" for protein products such as insulin.						