

CHAPTERS 16 & 17: DNA Technology

1. What is the function of restriction enzymes in bacteria?

2. How do bacteria protect their DNA from the effects of the restriction enzymes?

3. How do biologists make use of restriction enzymes?

4. How are DNA fragments of different sizes separated?

5. What does the technique of Southern Blotting accomplish?

6. What are some other techniques that build on the Southern Blotting technique?

7. What is meant by "recombinant DNA technology?"

Name: _____

Note Set 32

8. What are vectors?

9. What are plasmids?

10. What are reporter genes?

11. What is a genomic library?

12. How is cDNA different from typical eukaryote DNA?

13. What are knockout genes?

14. What are various ways that mRNA can control gene expression?

15. In the future, DNA chips may be used for regular diagnostics. What do the florescent spots indicate when the chip is read?

16. Define biotechnology.

17. What are other organisms used in biotechnology?

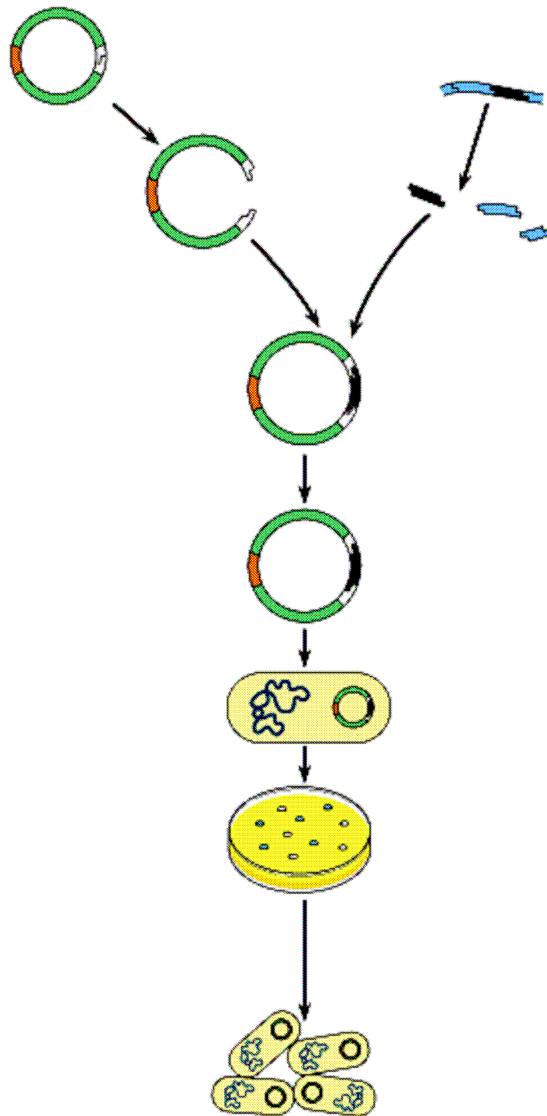
18. List some of the organisms we have been modifying for many hundreds of years.

19. Why are bacteria ideal workhorses for biotechnology?

20. How does gene cloning differ from human cloning?

21. Why is DNA cloning considered an important technology?

22. Describe the steps involved in cloning a gene.



23. What is 'pharming'?

24. What are some examples of GM Foods?

25. What technique has been used to modify agricultural plants?

26. List a few of the traits that have been engineered into agricultural plants? Could any of these pose an environmental threat?

27. What is a RFLP? How are they made? (Refer to section **17.2**)

28. What was the goal of the Human Genome Project?

29. List some of the most important things we learned by completing the Human Genome Project.

31. How has forensics made use of DNA technology? Give a specific example.

33. What is currently used by the FBI to do a DNA fingerprint in a criminal investigation?

END OF CHAPTER 16 MULTIPLE CHOICE

1. Restriction enzymes
 - A) play no role in bacteria.
 - B) cleave DNA at highly specific recognition sequences.
 - C) are inserted into bacteria by bacteriophage.
 - D) are made only by eukaryotic cells.
 - E) add methyl groups to specific DNA sequences.

2. When fragments of DNA of different sizes are placed in an electric field,
 - A) the smaller pieces move most rapidly toward the positive pole.
 - B) the larger pieces move most rapidly toward the positive pole.
 - C) the smaller pieces move most rapidly toward the negative pole.
 - D) the larger pieces move most rapidly toward the negative pole.
 - E) the smaller and larger pieces move at the same rate.

3. From the list below, select the sequence of steps for inserting a piece of foreign DNA into a plasmid vector, introducing the plasmid into bacteria, and verifying that the plasmid and the foreign gene are present:
 - (1) Transfect host cells.
 - (2) Select for the lack of plasmid reporter gene 1 function.
 - (3) Select for the plasmid reporter gene 2 function.
 - (4) Digest vector and foreign DNA with a restriction enzyme, which inactivates plasmid reporter gene 1.
 - (5) Ligate the digested plasmid together with the foreign DNA.
 - A) 45132
 - B) 45123
 - C) 13425
 - D) 32145
 - E) 13254

4. Possession of which feature is not desirable in a vector for gene cloning?
 - A) An origin of DNA replication
 - B) Genetic markers for the presence of the vector
 - C) Multiple recognition sequences for the restriction enzyme to be used
 - D) One recognition sequence each for one to several different restriction enzymes
 - E) Genes other than the target for transfection

5. RNA interference (RNAi) inhibits
 - A) DNA replication.
 - B) transcription of specific genes.
 - C) recognition of the promoter by RNA polymerase.
 - D) transcription of all genes.
 - E) translation of specific mRNAs.

6. Complementary DNA (cDNA)
 - A) is produced from ribonucleoside triphosphates.
 - B) is produced by reverse transcription.
 - C) is the "other strand" of single-stranded DNAs in a virus.
 - D) requires no template for its synthesis.
 - E) cannot be placed into a vector because it has the opposite base sequence of the vector DNA.

7. In a gene library of frog DNA in *E. coli* bacteria,
 - A) all bacterial cells have the same sequences of frog DNA.
 - B) all bacterial cells have different sequences of frog DNA.
 - C) each bacterial cell has a random fragment of frog DNA.
 - D) each bacterial cell has many fragments of frog DNA.
 - E) the frog DNA is transcribed into mRNA in the bacterial cells.

8. An expression vector requires all of the following except
 - A) genes for ribosomal RNA.
 - B) a reporter gene.
 - C) a promoter of transcription.
 - D) an origin of DNA replication.
 - E) restriction enzyme recognition sequences.

9. "Pharming" is a term that describes
 - A) the use of animals in transgenic research.
 - B) plants making genetically altered foods.
 - C) synthesis of recombinant drugs by bacteria.
 - D) large-scale production of cloned animals.
 - E) synthesis of a drug by a transgenic animal in its milk.

10. In DNA fingerprinting,
 - A) a positive identification can be made.
 - B) a gel blot is all that is required.
 - C) multiple restriction enzymes generate unique fragments.
 - D) the polymerase chain reaction amplifies finger DNA.
 - E) the variation in repeated sequences between two restriction sites is evaluated.

END OF CHAPTER 17 MULTIPLE CHOICE

11. Phenylketonuria is an example of a genetic disease in which
 - A) a single enzyme is not functional.
 - B) inheritance is sex-linked.
 - C) two parents without the disease cannot have a child with the disease.
 - D) mental retardation always occurs, regardless of treatment.
 - E) a transport protein does not work properly.

12. Mutations of the gene for β -globin
 - A) are usually lethal.
 - B) occur only at amino acid position 6.
 - C) number in the hundreds.
 - D) always result in sickling of red blood cells.
 - E) can always be detected by gel electrophoresis.

13. Multifactorial (complex) diseases
 - A) are less common than single-gene diseases.
 - B) involve the interaction of many genes with the environment.
 - C) affect less than 1 percent of humans.
 - D) involve the interactions of several mRNAs.
 - E) are exemplified by sickle-cell disease.

14. In fragile-X syndrome,
 - A) females are affected more severely than males.
 - B) a short sequence of DNA is repeated many times to create the fragile site.
 - C) both the X and Y chromosomes tend to break when prepared for microscopy.
 - D) all people who carry the gene that causes the syndrome are mentally retarded.
 - E) the basic pattern of inheritance is autosomal dominant.

15. Most genetic diseases are rare because
 - A) each person is unlikely to be a carrier for harmful alleles.
 - B) genetic diseases are usually sex-linked and so uncommon in females.
 - C) genetic diseases are always dominant.
 - D) two parents probably do not carry the same recessive alleles.
 - E) mutation rates in humans are low.

16. Mutational "hot spots" in human DNA
 - A) always occur in genes that are transcribed.
 - B) are common at cytosines that have been modified to 5-methylcytosine.
 - C) involve long stretches of nucleotides.
 - D) occur where there are long repeats.
 - E) are very rare in genes that code for proteins.

17. Newborn genetic screening for PKU
- A) is very expensive.
 - B) detects phenylketones in urine.
 - C) has not led to the prevention of mental retardation resulting from this disorder.
 - D) must be done during the first day of an infant's life.
 - E) uses bacterial growth to detect excess phenylalanine in blood.
18. Genetic diagnosis by DNA testing
- A) detects only mutant and not normal alleles.
 - B) can be done only on eggs or sperm.
 - C) involves hybridization to rRNA.
 - D) utilizes restriction enzymes and a polymorphic site.
 - E) cannot be done with PCR.
19. Most human cancers
- A) are caused by viruses.
 - B) are in blood cells or their precursors.
 - C) involve mutations of somatic cells.
 - D) spread through solid tissues rather than by the blood or lymphatic system.
 - E) are inherited.
20. Current treatments for genetic diseases include all of the following except
- A) restricting a dietary substrate.
 - B) replacing the mutant gene in all cells.
 - C) alleviating the patient's symptoms.
 - D) inhibiting a harmful metabolic reaction.
 - E) supplying a protein that is missing.

INSIGHTS LEARNED FROM THE SEQUENCE

What has been learned from analysis of the working draft sequence of the human genome? What is still unknown?*

*information taken from Science, Nature, Wellcome Trust, and Human Genome News

By the Numbers

- The human genome contains 3164.7 million chemical nucleotide bases (A, C, T, and G).
- The average gene consists of 3000 bases, but sizes vary greatly, with the largest known human gene being dystrophin at 2.4 million bases.
- The total number of genes is estimated at 30,000 to 35,000, much lower than previous estimates of 80,000 to 140,000 that had been based on extrapolations from gene-rich areas as opposed to a composite of gene-rich and gene-poor areas.
- The order of almost all (99.9%) nucleotide bases are exactly the same in all people.
- The functions are unknown for over 50% of discovered genes.

The Wheat from the Chaff

- Less than 2% of the genome encodes for the production of proteins.
- Repeated sequences that do not code for proteins ("junk DNA") make up at least 50% of the human genome.
- Repetitive sequences are thought to have no direct functions, but they shed light on chromosome structure and dynamics. Over time, these repeats reshape the genome by rearranging it, thereby creating entirely new genes or modifying and reshuffling existing genes.
- During the past 50 million years, a dramatic decrease seems to have occurred in the rate of accumulation of repeats in the human genome.

How It's Arranged

- The human genome's gene-dense "urban centers" are predominantly composed of the DNA building blocks G and C.
- In contrast, the gene-poor "deserts" are rich in the DNA building blocks A and T. GC- and AT-rich regions usually can be seen through a microscope as light and dark bands on chromosomes.
- Genes appear to be concentrated in random areas along the genome, with vast expanses of noncoding DNA between.
- Stretches of up to 30,000 C and G bases repeating over and over often occur adjacent to gene-rich areas, forming a barrier between the genes and the "junk DNA." These CpG islands are believed to help regulate gene activity.
- Chromosome 1 has the most genes (2968), and the Y chromosome has the fewest (231).

How the Human Genome Compares with That of Other Organisms

- Unlike the human's seemingly random distribution of gene-rich areas, many other organisms' genomes are more uniform, with genes evenly spaced throughout.
- Humans have on average three times as many kinds of proteins as the fly or worm because of mRNA transcript "alternative splicing" and chemical modifications to the proteins. This process can yield different protein products from the same gene.
- Humans share most of the same protein families with worms, flies, and plants, but the number of gene family members has expanded in humans, especially in proteins involved in development and immunity.
- The human genome has a much greater portion (50%) of repeat sequences than the mustard weed (11%), the worm (7%), and the fly (3%).
- Although humans appear to have stopped accumulating repeated DNA over 50 million years ago, there seems to be no such decline in rodents. This may account for some of the fundamental differences between hominids and rodents, although gene estimates are similar in these species. Scientists have proposed many theories to explain evolutionary contrasts between humans and other organisms, including those of life span, litter sizes, inbreeding, and genetic drift.

Variations and Mutations

- Scientists have identified about 1.4 million locations where single-base DNA differences (SNPs) occur in humans. This information promises to revolutionize the processes of finding chromosomal locations for disease-associated sequences and tracing human history.
- The ratio of germline (sperm or egg cell) mutations is 2:1 in males vs females. Researchers point to several reasons for the higher mutation rate in the male germline, including the greater number of cell divisions required for sperm formation than for eggs.

What We Still Don't Know: A Checklist for Future Research

- Exact gene number, exact locations, and functions
- Gene regulation
- DNA sequence organization
- Chromosomal structure and organization
- Noncoding DNA types, amount, distribution, information content, and functions
- Coordination of gene expression, protein synthesis, and post-translational events
- Interaction of proteins in complex molecular machines
- Predicted vs experimentally determined gene function
- Evolutionary conservation among organisms
- Protein conservation (structure and function)
- Proteomes (total protein content and function) in organisms
- Correlation of SNPs (single-base DNA variations among individuals) with health and disease
- Disease-susceptibility prediction based on gene sequence variation
- Genes involved in complex traits and multigene diseases