## 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)

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## 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**
October 14, 1946
(60 Minutes: Designing Life) (ABC: First Synthetic Cell)

# Guided Reading: Chapter 20

(p.375) **1.** It is important to understand the meaning of the three terms in below to start this chapter.

**(a) recombinant DNA –** DNA in which genes from two different sources - often different species - are combined in vitro (in a lab) into the same molecule

**(b) genetic engineering –** the direct manipulation of genes for practical purposes

**(c) biotechnology -** the manipulation of organisms or their components to make useful products

(p.376-77) **2.** ***Plasmids*** are important in biotechnology. Give a full and complete definition of ***plasmid***.

A plasmid are small, circular DNA molecules that replicate within bacterial cells.

(p.376) **3.** The production of multiple copies of a single gene is called gene cloning. (Animation)

(Animation: Producing Human Insulin)

(p.376) **4.** Using Figure 20.1, label and explain the five steps in this preview of ***gene cloning***.

(1) Isolation of plasmid DNA and DNA containing a gene of interest.

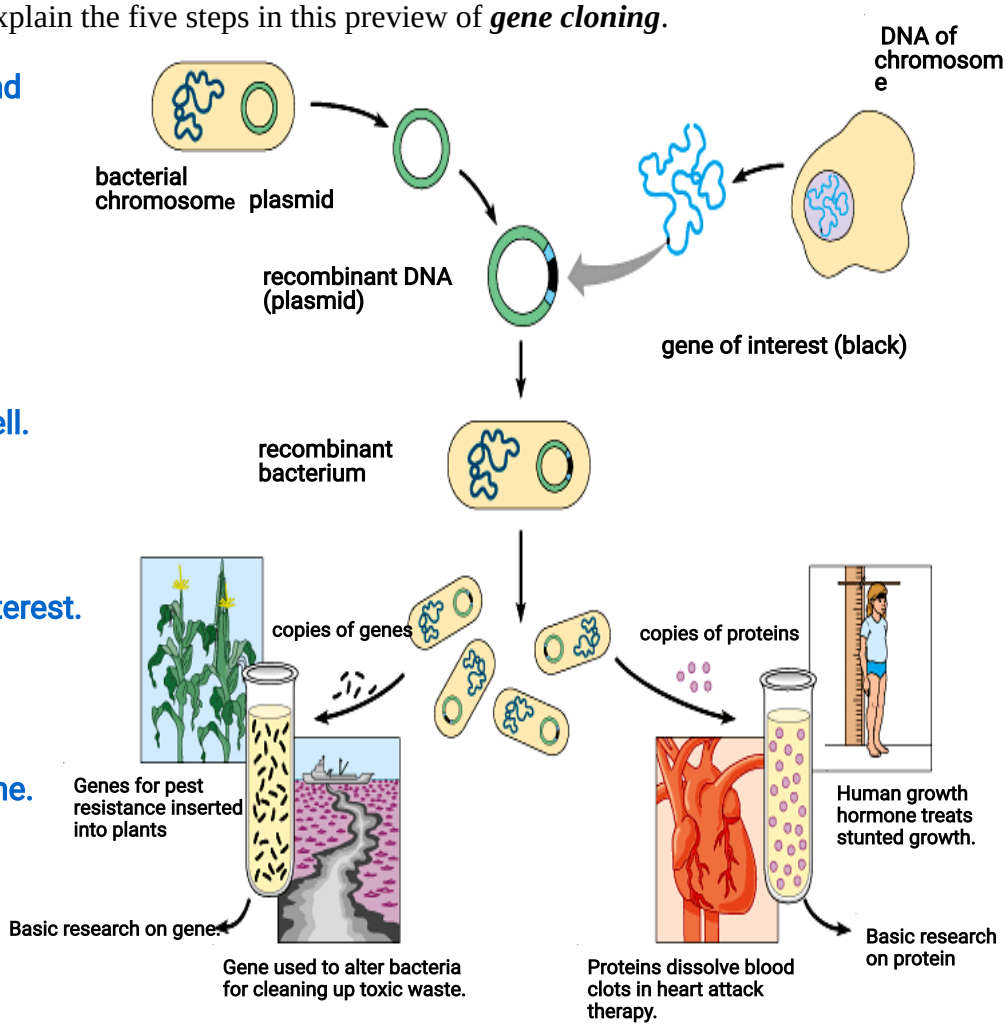
(2) Gene inserted into plasmid.

(3) Plasmid put into bacterial cell.

(4) Cells cloned with gene of interest.

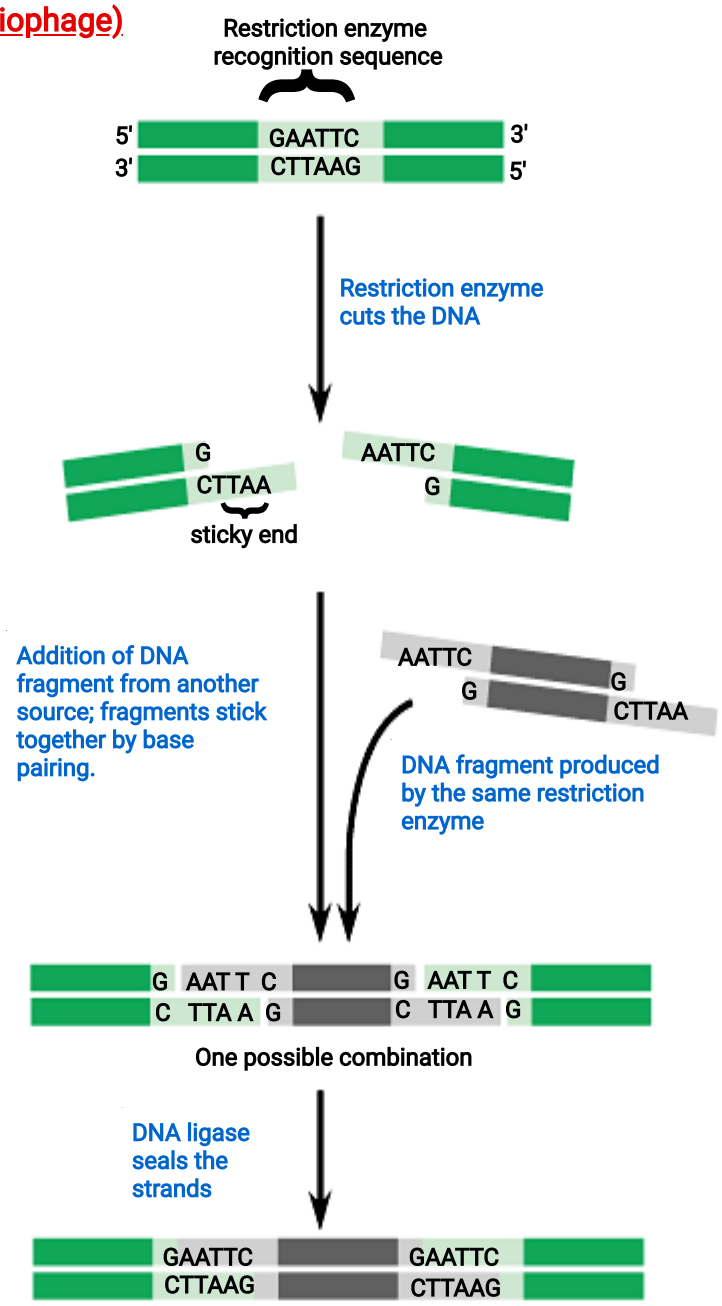
(5) Identification of desired clone.

(6) Various applications



(p.377) **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. (Animation)

(Animation: Bacteriophage)




(p.377) **6.** When were ***restriction enzymes*** discovered and what function do they serve in nature?

Restriction enzymes were discovered in the late 1960s by researchers studying bacteria. In nature, these enzymes protect bacteria against intruding DNA from other sources such as bacteriophages or other bacterial cells.

(p.379) **7.** What is a ***cloning vector***?

A cloning vector is defined as a DNA molecule that can carry foreign DNA into a cell and replicate there.

(p.379) **8.** Figure **20.3** is a more detailed discussion of the gene cloning procedure shown in Figure 20.1. Explain the following key points. (Animation)

**(a)** Explain why the plasmid is engineered with *ampR* gene.

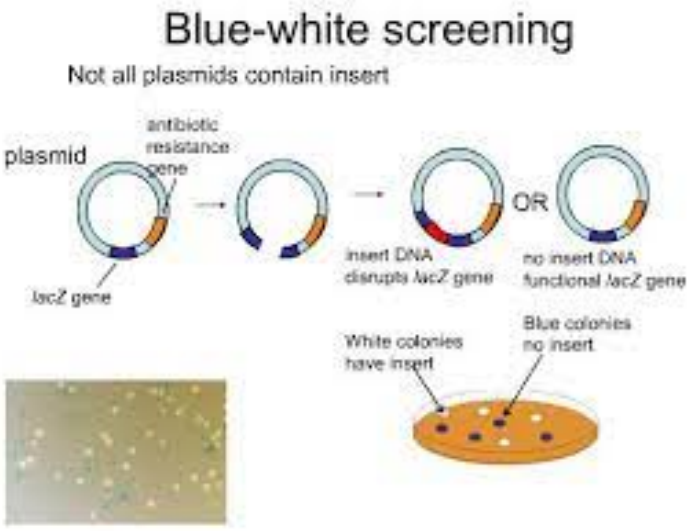
The plasmid is engineered with $amp^R$ gene which confers resistance to the antibiotic ampicillin so that later, when the cells are grown in a medium containing the antibiotic ampicillin, only the cells with the $amp^R$ gene can grow making it very easy to select the cells with the plasmid.

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

Some of the colonies are blue because along with the $amp^R$ gene, the plasmid is also engineered with a lacZ lactose gene encoding the enzyme B-galactosidase, which catalyzes the hydrolysis of the sugar lactose. Later, when the cells are grown in a medium containing the sugar X-gal, the enzyme B-galactosidase breaks down the sugar X-gal which produces a blue product, so bacterial colonies containing the plasmids with intact B-galactosidase genes (*gene not cut with restriction enzymes*) will turn blue.

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

Some of the colonies are white because those plasmids with foreign DNA inserted into its LacZ gene cannot produce B-galactosidase yielding cells that are white.



(p.379) **9.** Describe how a radioactively labeled nucleic acid probe can locate the gene of interest. (*Use Figure 20.4 to guide your response*.)

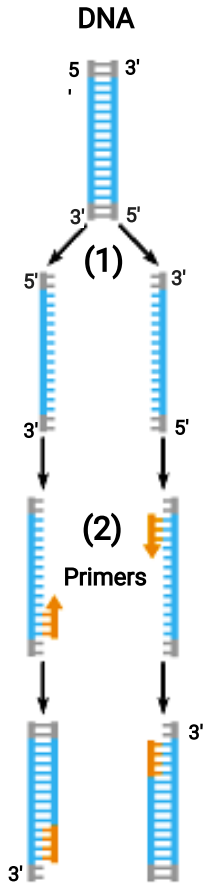
Radioactively labeled nucleic acid probes are short, single-stranded nucleic acids (DNA or RNA) that are complimentary to and bind with the gene of interest thus locating it.

(p.381)**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?

The purpose of a genomic library is to act as a source of other genes of interest or for gene mapping.

(Sumanas: PCR)

(p.382) **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)** Heat briefly to separate DNA strands.

**(2)** Cool to allow primers to hydrogen bond.

**(3)** DNA polymerase adds nucleotides to the 3' end of each of the primer.

(3)

(DNA Learning Center: PCR)

(p.382)**12.** How many molecules will be produced by four **PCR** cycles?

Four cycles will produce 16 molecules of DNA.

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.)*

(p.383) **13.** Gel electrophoresis is a technique used to separate nucleic acids or proteins that differ in ***size*** or ***electrical charge***.

(p.384) **14.** Why is the DNA sample to be separated by ***gel electrophoresis*** always loaded at the cathode or negative end of the power source?

DNA is always loaded at the cathode or negative end of the power source because DNA has a negative charge and you want it to migrate (move) towards the positive end.

**15.** Why is DNA treated with ***restriction enzymes*** before analyzed via gel electrophoresis.

DNA is treated with various restriction enzymes to produces DNA fragments of various sizes.

(p.386) **16.** What are ***restriction fragment length polymorphisms*** (*RFLP's*)?

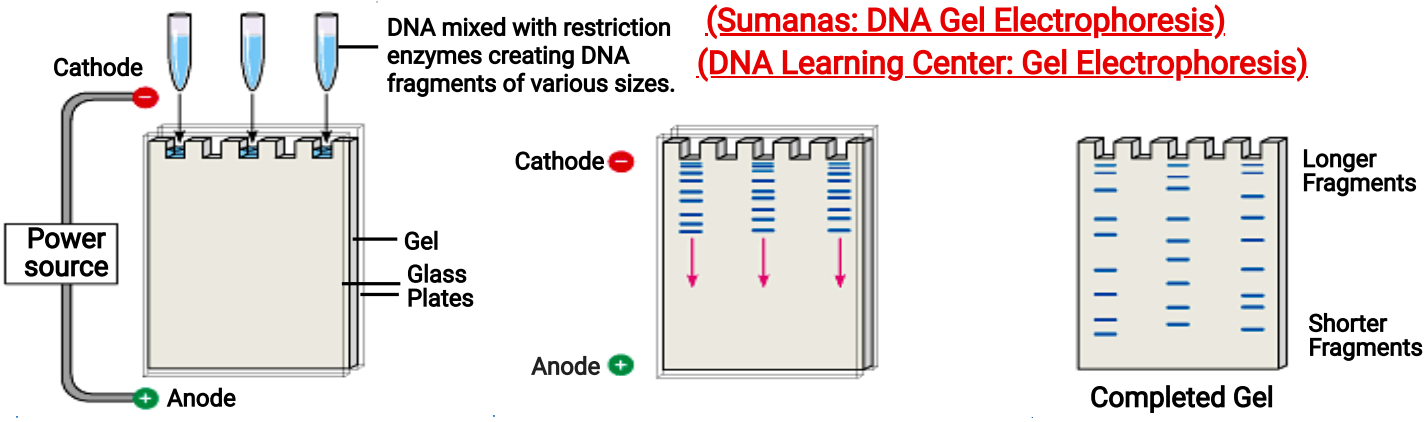
Restriction fragment length polymorphisms (RFLP's) are differences among individuals in the lengths of DNA fragments cut by restriction enzymes.

(p.385) **17.** Explain why shorter DNA fragments or *RFLP's* travel farther down the gel than larger molecules.

Shorter DNA fragments or RFLP's travel farther down the gel than larger molecules because they can move faster through the gel than larger fragment.

(p.384) **18.** Label the diagram below and use it to explain the process of ***cell electrophoresis***. (Learn Genetics Utah)

(Sumanas: DNA Gel Electrophoresis)
(DNA Learning Center: Gel Electrophoresis)




**(1)** Three samples, each containing a mixture of DNA molecules, are placed in wells near one end of the gel. The gel is supported by glass plates and bathed in an aqueous solution. Electrodes are attached at both ends, and voltage is applied.

**(2)** The DNA molecules, which are negatively charged, migrate or move to the positive electrode, the anode. A molecules rate of movement is determined mostly by its length; longer molecules travel more slowly through the gel.

**(3)** When the electrical current is turned off, the DNA molecules in each sample are arrayed in bands along a "lane" according to their size. The shortest molecule, having traveled the farthest, are the bands at the bottom of the gel.

(p.387) **19.** What is the ***Human Genome Project***?

The Human Genome Project was the international research effort to determine the DNA sequence of the entire human genome.

(p.390) **20.** What was one surprising result of the Human Genome Project?

One surprising result of the Human Genome Project was how little of the human genome is actually gene. The 25,000 genes was much lower than the estimated 50,000 to 100,000 genes expected.

(p.390) **21.** So what makes human - and vertebrate animals in general - more complex than flies or worms?

What makes human - and vertebrate animals in general - more complex than flies or worms is that our genomes seem to do more mixing and matching of modular elements - exons and protein domains.

(p.390) **22.** What is ***proteomics***?

Proteomics is the systematic study of the full protein sets (proteomes) encoded by genomes.

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

Humans (*Homo sapiens*) have a small amount of DNA variations compared to other species because Home sapiens are a relatively new species and enough time has not elapsed to create genetic variations in the species.

(p.392) **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)?

Single nucleotide polymorphisms are single base-pair variations found in a species genome and utilized during the DNA Gel electrophoreses process.

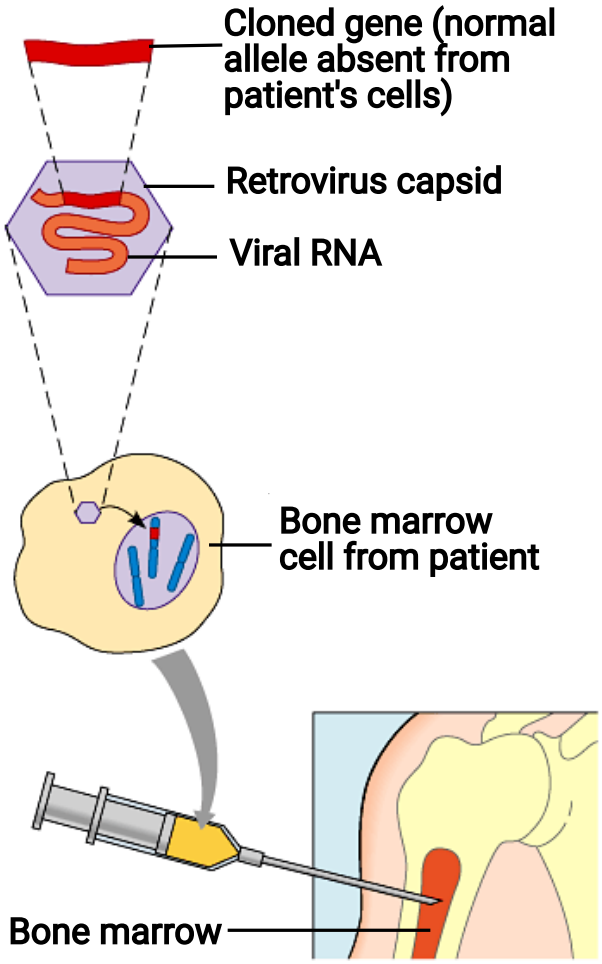
(p.393) **25.** How has DNA technology helped in the diagnosis and treatment of human genetic diseases?

DNA technology has helped in the diagnosis and treatment of human genetic diseases by identifying the gene whose mutations are responsible for such diseases leading to ways of diagnosing and treating them.

(p.393) **26.** What is gene therapy? (Animation-I) (Animation-II)

Gene therapy is the alteration of an afflicted individual's genes.

(p.394) **27.** Label the diagram below and use it to describe one type gene therapy.




(1) Insert RNA version of normal allele into retrovirus.

(2) Let retrovirus infect bone marrow cells that have been removed from the patient and cultured (grown in a lab).

(3) Viral DNA carrying the normal alleles inserts into chromosome.

(4) Inject engineered cells into patient.

(p.394) **28.** Why hasn't gene therapy proven to be very effective at correcting genetic defects in human somatic (*body cells*) cells?

Gene therapy hasn't proven to be very effective at correcting genetic defects in human somatic cells because even when genes are successfully and safely transferred and are being expressed in their new host cell, their activity typically diminishes after a short period.

(p.394) **29.** If not for correcting genetic defects, then what are researcher's currently using gene therapy for?

Researchers are currently using gene therapy in the fight against major killers such as heart disease and cancer.

(p.395) **30.** What was one of the first practical applications of gene splicing in the pharmaceutical industry? (Animation-I) (Animation-II)

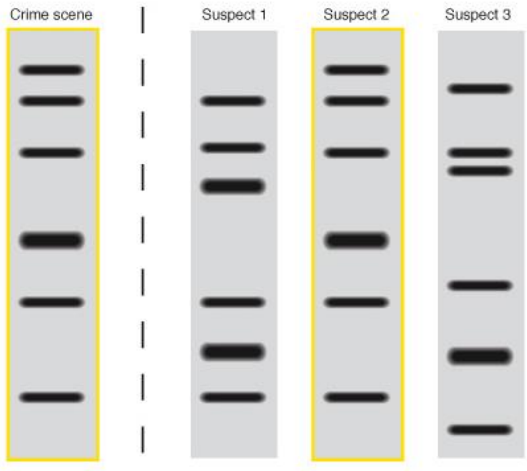
The first practical applications of gene splicing in the pharmaceutical industry in the development of "smart drugs."

(p.395) **31.** How do Forensic Scientists use RFLP analysis by Southern blotting to solve murder cases?

Forensic Scientists use RFLP analysis by Southern blotting to solve murder cases by comparing DNA samples from the suspect, the victim, and a small amount of blood found at the crime scene.

(p.396) **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)*

Suspect's 2 DNA matches the DNA found at the crime scene as revealed by the RFLP analysis and the locations of the 6 markers matching that of the crime scene and suspect 2.




(p.396) **33.** How is genetic engineering/DNA technology being applied to environmental work?

Genetic engineering/DNA technology has been applied to environmental work by genetically engineering microorganisms (bacteria) to capable of transforming or even removing harmful chemicals such as heavy metals, toxic waste and hydrocarbons (oil) from ecosystems.

(p.397) **34.** How is genetic engineering/DNA technology being used in agriculture, specifically animal husbandry?

Genetic engineering/DNA technology has been used in agriculture, specifically animal husbandry through the improvement of vaccines and growth hormones and the experimental production of transgenic animals, whose genomes carry genes from another species.

(p.397) **35.** How is genetic engineering/DNA technology being used in agriculture, specifically crop production?

Genetic engineering/DNA technology has been used in agriculture, specifically crop production by providing a number of plants with genes for desirable traits, such as delayed ripening and resistance to spoilage and disease.

(p.399) **36.** What are **G**enetically **M**odified **O**rganisms (GMO's)?

Genetically Modified Organisms (GMO's) are organisms that have acquired one or more means by artificial means.

(p.375) **37.** What are some of the major safety concerns relating to DNA technology?

Some of the major safety concerns relating to DNA technology

(p.375) **38.** What are some of the major ethical concerns relating to DNA technology?