Name _____ Period _____

Date _____

AP: CHAPTER 20: DNA TECHNOLOGY

1. Define biotechnology.

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

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

4. Why are bacteria ideal workhorses for biotechnology?

5. What are other organisms used in biotechnology?

6. How does gene cloning differ from human cloning?

Name _____

7. Why is DNA cloning considered an important technology?

_____

_____

8. What are plasmids?

_____

_____

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

_____

_____

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

_____

_____

11. How do biologists make use of restriction enzymes?

_____

_____

12. What is a genomic library?

_____

_____

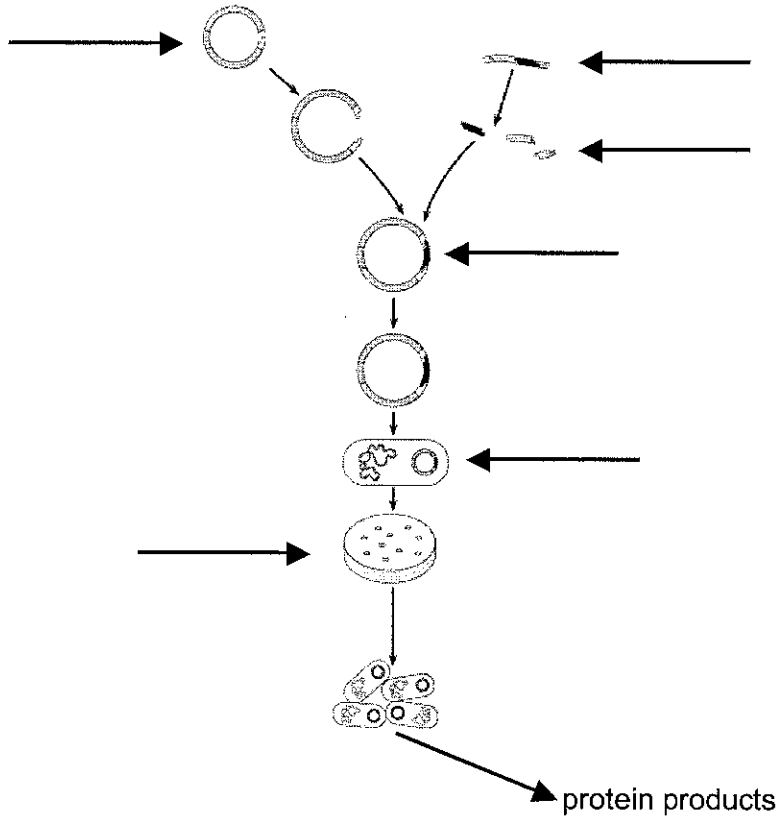
13. How is cDNA different from typical eukaryote DNA?

_____

_____

Name _____

14. Describe the steps involved in cloning a gene.



15. How can transformed bacteria that carry genes of interest be identified and isolated from the majority of non-transformed bacteria?

_____

_____

_____

16. What can be accomplished with Nucleic Acid Hybridization?

_____

_____

_____

Name _____

17. What is the purpose of the Polymerase Chain Reaction?

_____

_____

18. List some advantages & uses of the PCR technique.

_____

_____

_____

19. How are DNA fragments of different sizes separated?

_____

_____

20. What is a RFLP? How are they made?

_____

_____

_____

_____

21. What does the technique of Southern Blotting accomplish?

_____

_____

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

_____

_____

Name _____

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

_____

_____

_____

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

_____

_____

25. What is the Sanger Sequencing Method used for?

_____

_____

26. How does the shot-gun approach differ from the whole-genome sequencing?

_____

_____

_____

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

_____

_____

28. How can DNA technology be used to diagnose a carrier of a genetic disorder?

_____

_____

Name _____

29. What is the goal of gene therapy?

_____

_____

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

_____

_____

_____

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

_____

_____

_____

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

_____

_____

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

_____

_____

_____

Name _____

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.

Name _____

- 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)

Name _____

- 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
- Complex systems biology, including microbial consortia useful for environmental restoration
- Developmental genetics, genomics

http://genome.gsc.riken.go.jp/hgmis/project/journals/insights.html

Restriction Enzymes

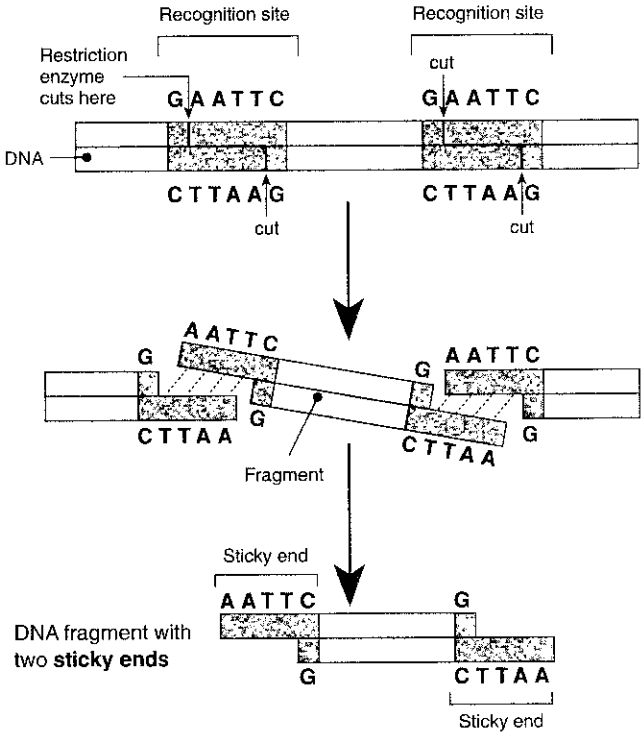
One of the essential tools of genetic engineering is a group of special **restriction enzymes** (also known as restriction endonucleases). These have the ability to cut DNA molecules at very precise sequences of 4 to 8 base pairs called **recognition sites**. These enzymes are the "molecular scalpels" that allow genetic engineers to cut up DNA in a controlled way. Although first isolated in 1970, these enzymes were discovered earlier in many bacteria (see panel on the next page). The purified forms of these bacterial restriction enzymes are used today as tools to cut DNA (see table on the next page for examples). Enzymes are named according to the bacterial species from which they were first isolated. By using a 'tool kit' of over 400 restriction enzymes recognizing about 100 recognition sites, genetic engineers can isolate, sequence, and manipulate individual genes derived from any type of organism. The sites at which the fragments of DNA are cut may result in overhanging "sticky ends" or non-overhanging "blunt ends". Pieces may later be joined together using an enzyme called **DNA ligase** in a process called **ligation**.

Sticky End Restriction Enzymes

1. A **restriction enzyme** cuts the double-stranded DNA molecule at its specific **recognition site** (see the table opposite for a representative list of restriction enzymes and their recognition sites).

2. The cuts produce a DNA fragment with two **sticky ends** (ends with exposed nucleotide bases at each end). The piece it is removed from is also left with sticky ends.

Restriction enzymes may cut DNA leaving an overhang or sticky end, without its complementary sequence opposite. DNA cut in such a way is able to be joined to other exposed end fragments of DNA with matching sticky ends. Such joins are specific to their recognition sites.

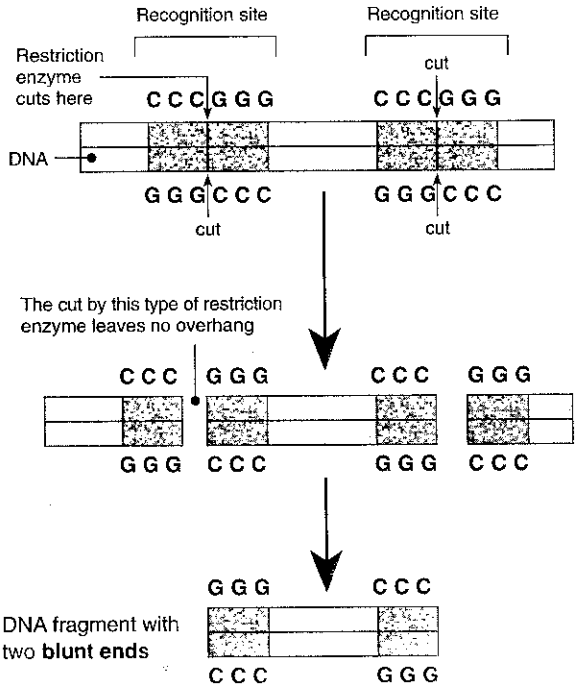


Blunt End Restriction Enzymes

1. A **restriction enzyme** cuts the double-stranded DNA molecule at its specific **recognition site** (see the table opposite for a representative list of restriction enzymes and their recognition sites).

2. The cuts produce a DNA fragment with two **blunt ends** (ends with no exposed nucleotide bases at each end). The piece it is removed from is also left with blunt ends.

It is possible to use restriction enzymes that cut leaving no overhang. DNA cut in such a way is able to be joined to any other blunt end fragment, but tends to be nonspecific because there are no sticky ends as recognition sites.



Origin of Restriction Enzymes

Restriction enzymes have been isolated from many bacteria. It was observed that certain *bacteriophages* (viruses that infect bacteria) could not infect bacteria other than their usual hosts. The reason was found to be that other potential hosts could destroy almost all of the phage DNA using *restriction enzymes* present naturally in their cells; a defense mechanism against the entry of foreign DNA. Restriction enzymes are named according to the species they were first isolated from, followed by a number to distinguish different enzymes isolated from the same organism.

Recognition sites for selected restriction enzymes

Enzyme	Source	Recognition Sites
***Eco*RI**	*Escherichia coli* RY13	G A A T T C
***Bam*HI**	*Bacillus amyloliquefaciens* H	G G A T C C
***Hae*III**	*Haemophilus aegyptius*	G G C C
***Hind*III**	*Haemophilus influenzae* Rd	A A G C T T
***Hpa*I**	*Haemophilus parainfluenzae*	G T T A A C
***Hpa*II**	*Haemophilus parainfluenzae*	C C G G
***Mbo*I**	*Moraxella bovis*	G A T C
***Not*I**	*Norcardia otitidis-caviarum*	G C G G C C G C
***Taq*I**	*Thermus aquaticus*	T C G A

1. Explain the following terms, identifying their role in recombinant DNA technology:

   (a) Restriction enzyme: _____

   _____

   (b) Recognition site: _____

   _____

   (c) Sticky end: _____

   _____

   (d) Blunt end: _____

   _____

2. The action of a specific sticky end restriction enzyme is illustrated on the previous page (top). Use the table above to:

   (a) Name the **restriction enzyme** used: _____

   (b) Name the organism from which it was first isolated: _____

   (c) State the **base sequence** for this restriction enzyme's recognition site: _____

3. A genetic engineer wants to use the restriction enzyme ***Bam*HI** to cut the DNA sequence below:

   (a) Consult the table above and state the recognition site for this enzyme: _____

   (b) Circle every **recognition site** on the DNA sequence below that could be cut by the enzyme ***Bam*HI**:

```
                10                  20                  30                  40                  50                  60
|AATGGGTACG|CACAGTGGAT|CCACGTAGTA|TGCGATGCGT|AGTGTTTATG|GAGAGAAGAA|
                70                  80                  90                 100                 110                 120
|AACGCGTCGC|CTTTTATCGA|TGCTGTACGG|ATGCGGAAGT|GGCGATGAGG|ATCCATGCAA|
               130                 140                 150                 160                 170                 180
|TCGCGGCCGA|TCGXGTAATA|TATCGTGGCT|GCGTTTATTA|TCGTGACTAG|TAGCAGTATG|
               190                 200                 210                 220                 230                 240
|CGATGTGACT|GATGCTATGC|TGACTATGCT|ATGTTTTTAT|GCTGGATCCA|GCGTAAGCAT|
               250                 260                 270                 280                 290                 300
|TTCGCTGCGT|GGATCCCATA|TCCTTATATG|CATATATTCT|TATACGGATC|GCGCACGTTT|
```

   (c) State how many fragments of DNA were created by this action: _____

4. When restriction enzymes were first isolated in 1970 there were not many applications to which they could be put to use. They are now an important tool in genetic engineering. Describe the human needs and demands that have driven the development and use of restriction enzymes in genetic engineering:

   _____

   _____

   _____

Ligation

DNA fragments produced using restriction enzymes may be reassembled by a process called **ligation**. Pieces are joined together using an enzyme called **DNA ligase**. DNA of different origins produced in this way is called **recombinant DNA** (because it is DNA that has been recombined from different sources). The combined techniques of using restriction enzymes and ligation are the basic tools of genetic engineering (also known as recombinant DNA technology).

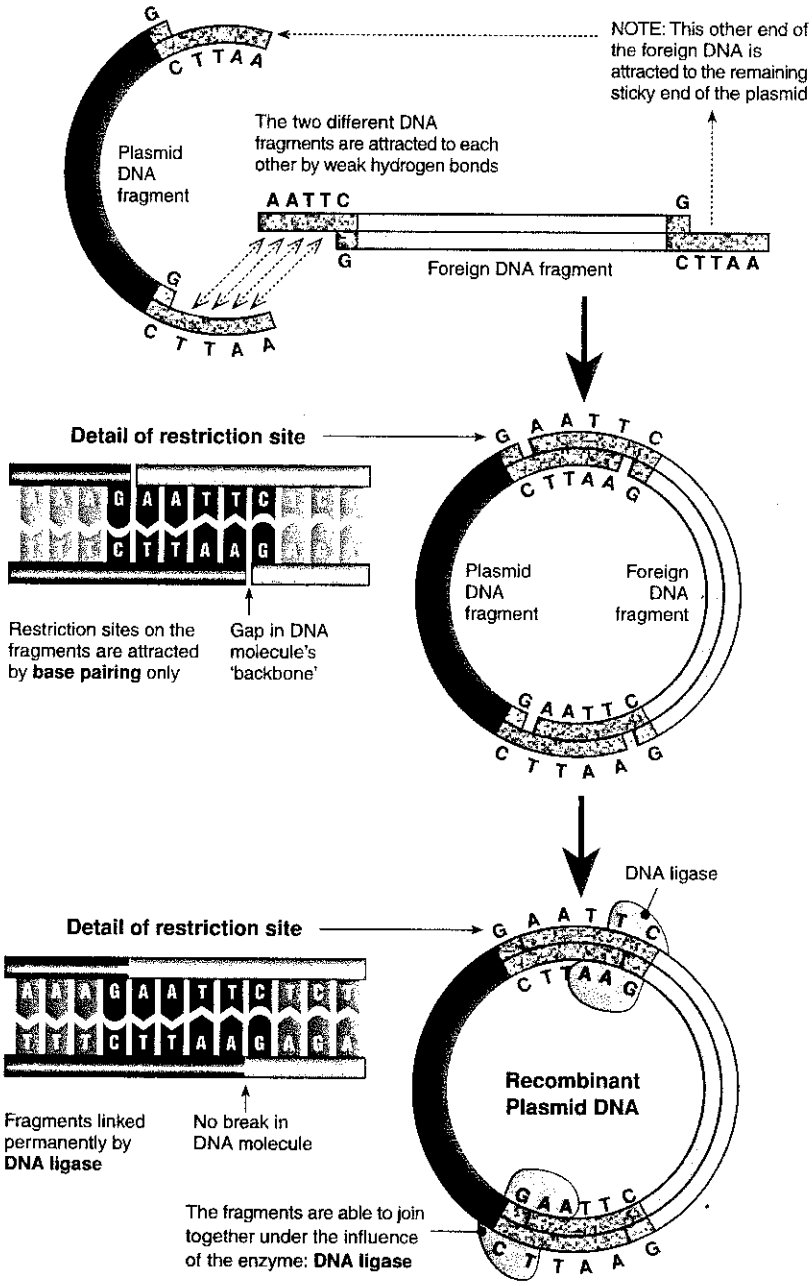
Creating a Recombinant DNA Plasmid

1. If two pieces of DNA are cut by the same restriction enzyme, they will produce fragments with matching **sticky ends** (ends with exposed nucleotide bases at each end).

2. When two such matching sticky ends come together, they can join by base-pairing. This process is called **annealing**. This can allow DNA fragments from a different source, perhaps a **plasmid**, to be joined to the DNA fragment.

3. The joined fragments will usually form either a linear molecule or a circular one, as shown here for a **plasmid**. However, other combinations of fragments can occur.

4. The fragments of DNA are joined together by the enzyme **DNA ligase**, producing a molecule of **recombinant DNA**.



1. Explain in your own words the two main steps in the process of joining two DNA fragments together:

   (a) Annealing: _____

   _____

   (b) DNA ligase: _____

   _____

2. Refer to the activity *DNA Replication* and briefly describe the usual role of DNA ligase in a cell: _____

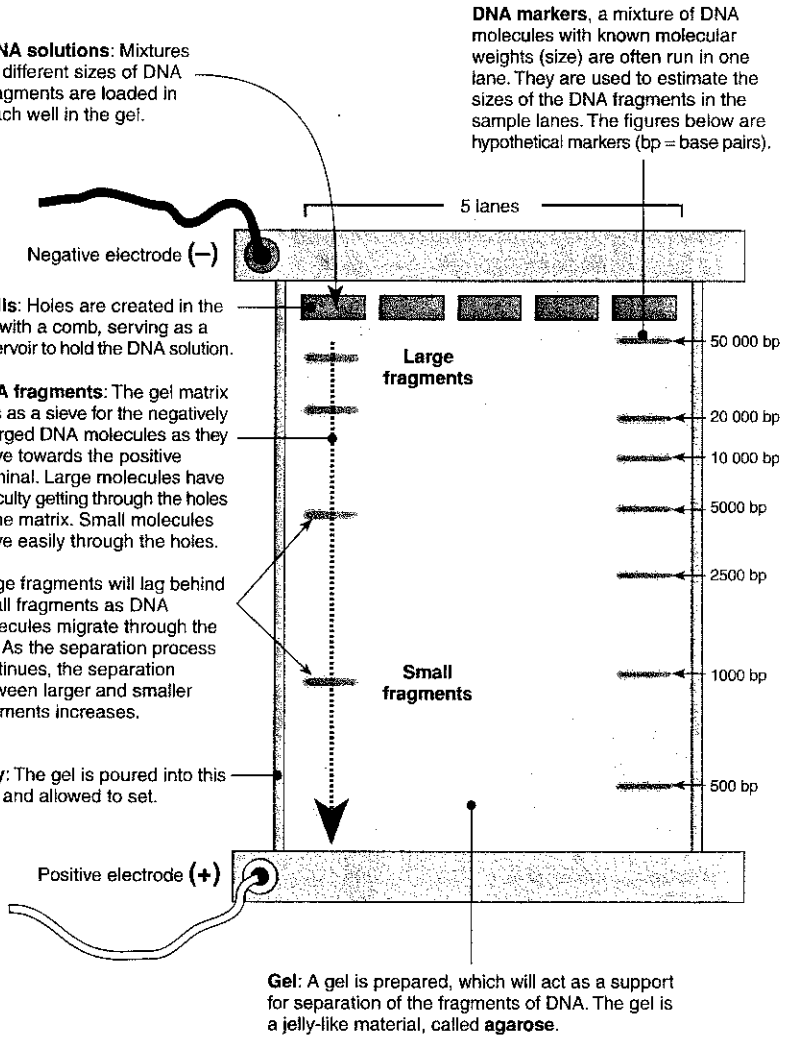
   _____

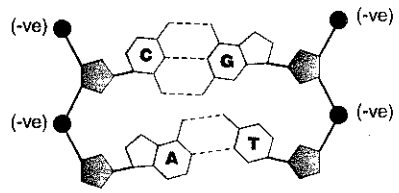
3. Explain why ligation can be considered the *reverse* of the restriction enzyme process: _____

Gel Electrophoresis

Gel electrophoresis is a method that separates large molecules (including nucleic acids or proteins) on the basis of size, electric charge, and other physical properties. Such molecules possess a slight electric charge (see DNA below). To prepare DNA for gel electrophoresis the DNA is often cut up into smaller pieces. This is done by mixing DNA with restriction enzymes in controlled conditions for about an hour. Called **restriction digestion**, it produces a range of DNA fragments of different lengths. During electrophoresis, molecules are forced to move through the pores of a **gel** (a jelly-like material), when the electrical current is applied. Active electrodes at each end of the gel provide the driving force. The electrical current from one electrode repels the molecules while the other electrode simultaneously attracts the molecules. The frictional force of the gel material resists the flow of the molecules, separating them by size. Their rate of migration through the gel depends on the strength of the electric field, size and shape of the molecules, and on the ionic strength and temperature of the buffer in which the molecules are moving. After staining, the separated molecules in each lane can be seen as a series of bands spread from one end of the gel to the other.

Analyzing DNA using Gel Electrophoresis





DNA is negatively charged because the phosphates (black) that form part of the backbone of a DNA molecule have a negative charge.

Steps in gel electrophoresis of DNA

1. A tray is prepared to hold the gel matrix.
2. A gel comb is used to create holes in the gel. The gel comb is placed in the tray.
3. Agarose gel powder is mixed with a buffer solution (the liquid used to carry the DNA in a stable form). The solution is heated until dissolved and poured into the tray and allowed to cool.
4. The gel tray is placed in an electrophoresis chamber and the chamber is filled with buffer, covering the gel. This allows the electric current from electrodes at either end of the gel to flow through the gel.
5. DNA samples are mixed with a "loading dye" to make the DNA sample visible. The dye also contains glycerol or sucrose to make the DNA sample heavy so that it will sink to the bottom of the well.
6. A safety cover is placed over the gel, electrodes are attached to a power supply and turned on.
7. When the dye marker has moved through the gel, the current is turned off and the gel is removed from the tray.
8. DNA molecules are made visible by staining the gel with ethidium bromide which binds to DNA and will fluoresce in UV light.

1. Explain the purpose of gel electrophoresis: _____

_____

_____

2. Describe the two forces that control the speed at which fragments pass through the gel:

   (a) _____

   (b) _____

3. Explain why the smallest fragments travel through the gel the fastest: _____

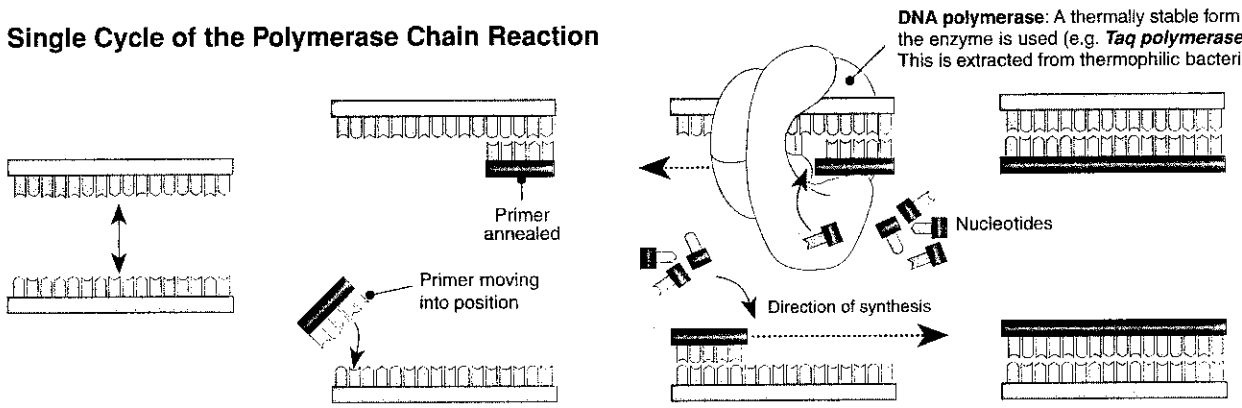
_____

_____

Polymerase Chain Reaction

Many procedures in DNA technology (such as DNA sequencing and DNA profiling) require substantial amounts of DNA to work with. Some samples, such as those from a crime scene or fragments of DNA from a long extinct organism, may be difficult to get in any quantity. The diagram below describes the laboratory technique called **polymerase chain reaction (PCR)**. Using this technique, vast quantities of DNA identical to trace samples can be created. This process is often termed **DNA amplification**. Although only one cycle of replication is shown below, following cycles replicate DNA at an exponential rate. PCR can be used to make literally billions of copies in only a few hours. **Linear PCR** differs from regular PCR in that the same original DNA templates are used repeatedly. It is used to make many radio-labeled DNA fragments for DNA sequencing.

A Single Cycle of the Polymerase Chain Reaction



1. A DNA sample (called target DNA) is obtained. It is **denatured** (DNA strands are separated) by heating at 98°C for 5 minutes.
2. The sample is cooled to 60°C. Primers are **annealed** (bonded) to each DNA strand. In PCR, the primers are short strands of DNA; they provide the starting sequence for DNA extension.
3. Free nucleotides and the enzyme DNA polymerase are added. DNA polymerase binds to the primers and, using the free nucleotides, synthesizes complementary strands of DNA.
4. After one cycle, there are now two copies of the original DNA.

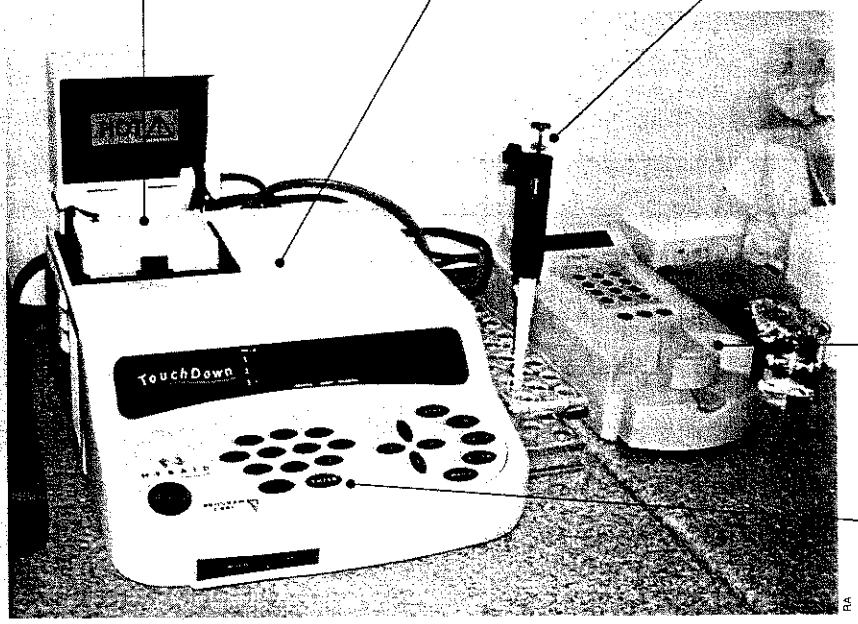
Repeat for about 25 cycles

Repeat cycle of heating and cooling until enough copies of the target DNA have been produced

Loading tray
Prepared samples in tiny PCR tubes are placed in the loading tray and the lid is closed.

Temperature control
Inside the machine are heating and refrigeration mechanisms to rapidly change the temperature.

Dispensing pipette
Pipettes with disposable tips are used to dispense DNA samples into the PCR tubes.



Thermal Cycler

Amplification of DNA can be carried out with simple-to-use machines called **thermal cyclers**. Once a DNA sample has been prepared, in just a few hours the amount of DNA can be increased billions of times. Thermal cyclers are in common use in the biology departments of universities, as well as other kinds of research and analytical laboratories. The one pictured on the left is typical of this modern piece of equipment.

DNA quantitation
The amount of DNA in a sample can be determined by placing a known volume in this quantitation machine. For many genetic engineering processes, a minimum amount of DNA is required.

Controls
The control panel allows a number of different PCR programs to be stored in the machine's memory. Carrying out a PCR run usually just involves starting one of the stored programs.

1. Explain the purpose of PCR: _____

_____

_____

2. Briefly describe how the **polymerase chain reaction** (PCR) works: _____

3. Describe three situations where only minute DNA samples may be available for sampling and PCR could be used:

   (a) _____

   (b) _____

   (c) _____

4. After only two cycles of replication, four copies of the double-stranded DNA exist. Calculate how much a DNA sample will have increased after:

   (a) 10 cycles: _____ (b) 25 cycles: _____

5. The risk of contamination in the preparation for PCR is considerable.

   (a) Explain what the effect would be of having a single molecule of unwanted DNA in the sample prior to PCR:

   (b) Describe two possible sources of DNA contamination in preparing a PCR sample:

   Source 1: _____

   Source 2: _____

   (c) Describe two precautions that could be taken to reduce the risk of DNA contamination:

   Precaution 1: _____

   Precaution 2: _____

6. Describe two other genetic engineering/genetic manipulation procedures that require PCR amplification of DNA:

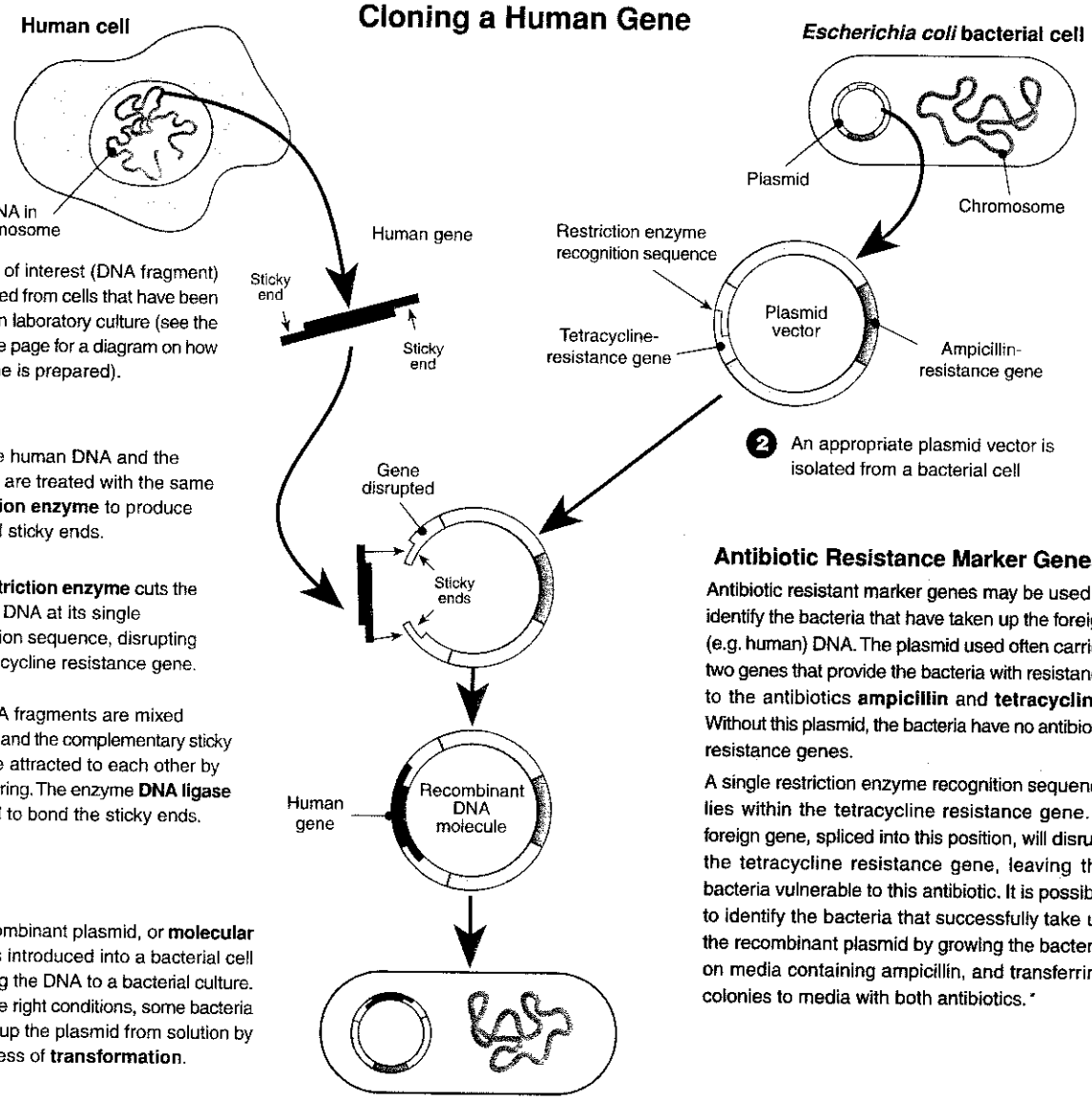
   (a) _____

   (b) _____

Gene Cloning Using Plasmids

Gene cloning is a process of making large quantities of a desired piece of DNA once it has been isolated. The purpose of this process is often to yield large quantities of either an individual gene or its protein product when the gene is expressed. Methods have been developed to insert a DNA fragment of interest (e.g. a human gene for a desired protein) into the DNA of a vector, resulting in a **recombinant DNA molecule** or **molecular clone**. A **vector** is a self-replicating DNA molecule (e.g. plasmid or viral DNA) used to transmit a gene from one organism into another. To be useful, all vectors must be able to replicate inside their host organism, they must have one or more sites at which a restriction enzyme can cut, and they must have some kind of **genetic marker** that allows them to be easily identified. Organisms such as bacteria, viruses and yeasts have DNA that behaves in this way. Large quantities of the desired gene can be obtained if the recombinant molecule is allowed to replicate in an appropriate host. The host (e.g. bacterium) may then go on to express the gene and produce the desired protein. Two types of vector are **plasmids** (illustrated below) and **bacteriophages** (viruses that infect bacteria).

Cloning a Human Gene



1. A gene of interest (DNA fragment) is isolated from cells that have been grown in laboratory culture (see the opposite page for a diagram on how the gene is prepared).

2. An appropriate plasmid vector is isolated from a bacterial cell

3. Both the human DNA and the plasmid are treated with the same **restriction enzyme** to produce identical sticky ends.

4. The **restriction enzyme** cuts the plasmid DNA at its single recognition sequence, disrupting the tetracycline resistance gene.

5. The DNA fragments are mixed together and the complementary sticky ends are attracted to each other by base-pairing. The enzyme **DNA ligase** is added to bond the sticky ends.

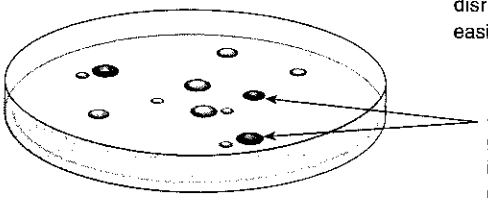
6. The recombinant plasmid, or **molecular clone**, is introduced into a bacterial cell by adding the DNA to a bacterial culture. Under the right conditions, some bacteria will take up the plasmid from solution by the process of **transformation**.

Preparation of the clone up to this point

Cloning the gene starts here

7. The actual gene cloning process (making multiple copies of the human gene) occurs when the bacterium with the recombinant plasmid is allowed to reproduce.

8. Colonies of bacteria that carry the recombinant plasmid can be identified by the fact that they are resistant to ampicillin but sensitive to tetracycline.



Agar plate with colonies of bacteria growing on them. All colonies look identical but only some have the plasmid with the human gene.

Antibiotic Resistance Marker Genes

Antibiotic resistant marker genes may be used to identify the bacteria that have taken up the foreign (e.g. human) DNA. The plasmid used often carries two genes that provide the bacteria with resistance to the antibiotics **ampicillin** and **tetracycline**. Without this plasmid, the bacteria have no antibiotic resistance genes.

A single restriction enzyme recognition sequence lies within the tetracycline resistance gene. A foreign gene, spliced into this position, will disrupt the tetracycline resistance gene, leaving the bacteria vulnerable to this antibiotic. It is possible to identify the bacteria that successfully take up the recombinant plasmid by growing the bacteria on media containing ampicillin, and transferring colonies to media with both antibiotics.

NOTE: Most often today, another gene plays the role of the tetracycline resistance gene, but the principle remains the same; the inserted DNA disrupts the activity of a gene whose activity is easily determined.