Biotech

Recombinant DNA technology (genetic engineering) involves combining genes from different sources into new cells that can express the genes.

Recombinant DNA technology has had-and will have-many important applications

- More efficient methods of basic and applied research into molecular genetics.
- Mass production by bacteria of biochemicals needed by other species.
- Creation of new strains of plant and animals.

Recombinant DNA Techniques are based on bacterial mechanisms

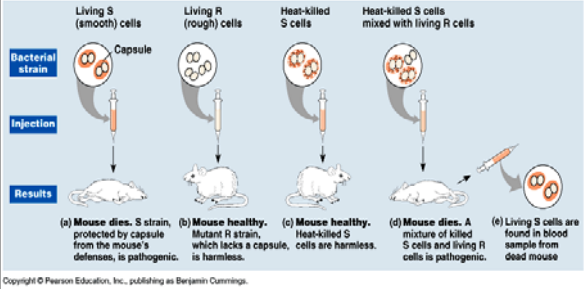
- In 1946, Ledberg & Tatum discovered that *E. Coli* has a sexual mechanism.
- They combined *E. Coli* strains, each of which required a different amino acid to grow. Cells of a new strain appeared in the cultures that did not require the addition of either amino acid.

Bacteria as Tools for Manipulating DNA

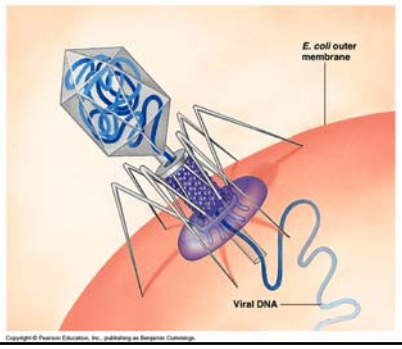
In nature bacteria can transfer DNA in three ways

- In sexually reproducing organisms, new genetic combinations are the result of meiosis and fertilization.
- Studies by Griffith showed nonpneumonia-causing stains of *Pnuemococcus* become disease causing in culture medium that previously contained the disease causing strain.

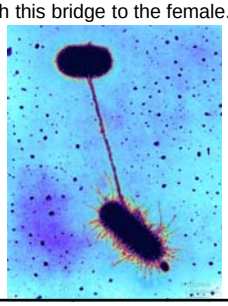
•**Transformation** is the taking up of DNA from the nonliving environment around a bacterium. Transformation caused the results Griiffith observed.



•**Transduction** is the transfer of bacterial genes from one bacterium to another by a phage.



•**Conjugation** is the process by which two bacteria mate. Conjugation is initiated by "male" cells (gene donors) that recognize "female" cells (gene recipients) by means of male sex pili. After initial male-female recognition, a cytoplasmic bridge forms between two cells. Replicated DNA from the male passes through this bridge to the female.



•In all three mechanisms, the new DNA is integrated into the existing DNA in the recipient by a cross-over like event that replace part of the existing DNA.

•These mechanisms are not reproductive. Sexual reproduction doe not occur in bacteria, unlike the situation in plants and animals.

Bacterial plasmids can serve as carriers for gene transfer

•The F (fertility) factor is a portion of *E. coli* DNA that carries genes for making sex pili and other requirements for conjugation.

•The F factor may be integrated into the main bacterial DNA or it may exist as a separate, circular DNA fragment, a **plasmid**, that is free in the cytoplasm. Plasmids replicate separately from the main DNA.

•If the F factor is integrated into the donor's main DNA, replication begins. The replicated length of DNA is transferred from the donor to the recipient but usually breaks before the remaining F factor is transferred. Thus the recipient does not receive the F-factor genes, and its descendants remain female.

•If the F factor exists as a separate plasmid, it replicates into a linear DNA molecule that is entirely transferred to the recipient. The recipient and all its descendants become male.

•When extra genes are transferred the plasmid is acting as a vector.

•Plasmids that carry genes other than those needed for conjugation are called **vectors**.

i.e.- R plasmids are a class of plasmids that carry genes for antibiotic resistance. The wide spread use of antibiotics in medicine and agriculture has tended to kill bacteria that lack the R plasmids and favor the bacteria that have R plasmids.

Plasmids are used to customize bacteria

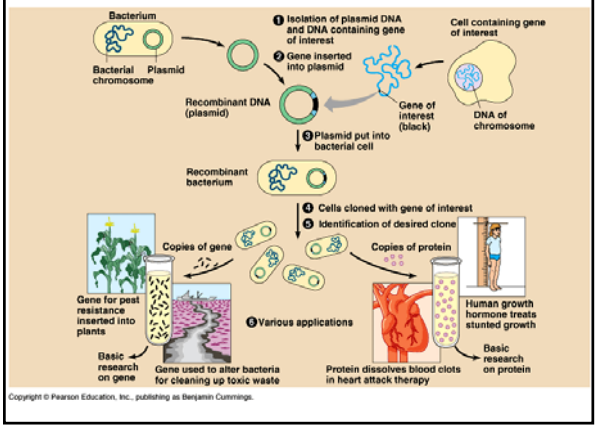
•Plasmids are isolated from a bacterium.

•DNA that encodes useful proteins or traits is removed from another organism.

•The plasmid DNA and gene of interest ar joined and returned to the bacterial cells.

•The bacteria are grown in culture to produce many copies of the isolated gene (the gene is cloned) or its product.

•Such engineered bacteria play a role in the manufacture of drugs such as **human insulin** and **human growth hormone**.



Enzymes are used to "cut and paste" DNA

•Restriction enzymes were first discovered in bacteria in the late 1960s.

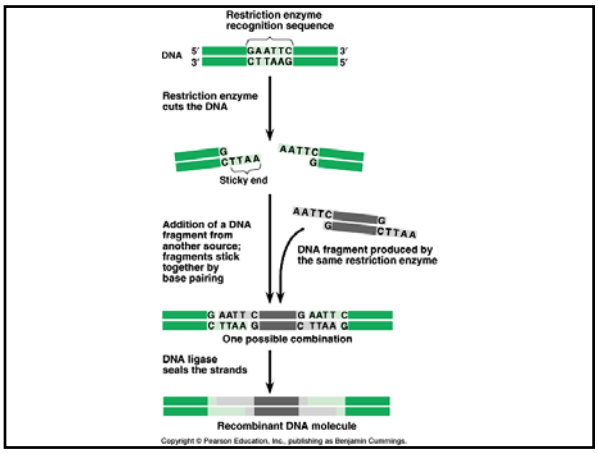
•In nature, bacteria use restriction enzymes to cut up intruder DNA from phages and from other organisms into nonfunctional pieces. The bacteria first chemically modify their own DNA so that it will not be cut.

•Several hundred different restriction enzymes have been discovered that recognize about 100 different sequences.

•DNA from two different sources is cut by the same restriction enzyme. These enzymes each cut at a specific restriction-enzyme recognition sequences.

•The result is double-stranded DNA sequences with single-stranded sticky ends.

•DNA fragments may pair at their sticky ends. This pairing is temporary but DNA ligase can make it permanent. The result of this is the formation of recombinant DNA.



Genes can be cloned in recombinant plasmids

•Plasmid DNA and the DNA of the cell containing the gene of interest are each cut with the same restriction enzyme.

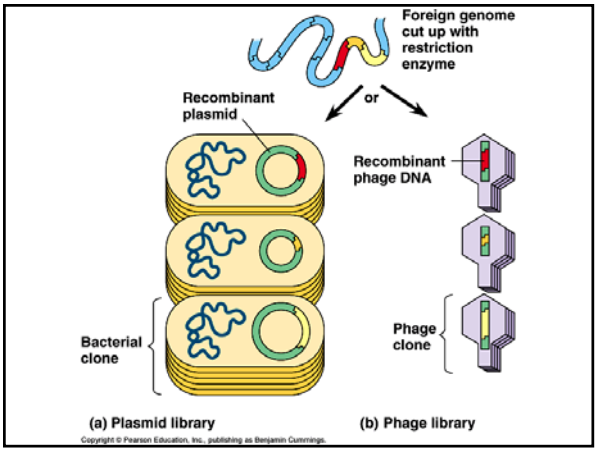
•The new gene is inserted into the plasmid. The new plasmid is returned to a bacterium by transformation.

•Scientists often used a "**shotgun**" approach since the specific gene isn't targeted.

Cloned genes can be stored in genomic libraries

•Using a shotgun approach to do this, scientists cut up target DNA into thousands of fragments, each of which carries a few genes of unknown identity (one or more fragments will carry genes of interest).

•These fragments are temporarily stored in genomic libraries of plasmids in separate bacterial cells, or in separate phages.



Other tools of DNA Technology

Reverse Transcriptase helps make genes for cloning

•A problem with cloning and bacterial synthesis of eukaryotic gene products is that bacterial genes do not contain introns.

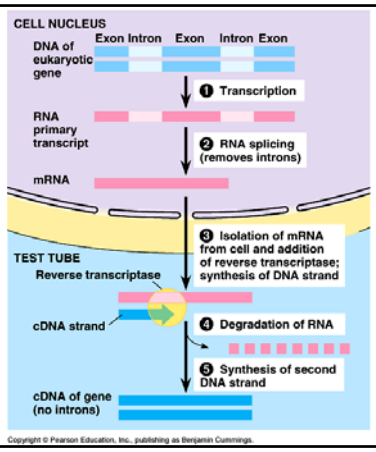
•Special enzymes called reverse transcriptase are found in retroviruses. These enzymes make DNA from viral genome RNA.

i.e.- HIV is a retrovirus

•Genes that are expressed can be isolated by using mRNA that has already had its introns spliced out. When reverse transcriptase is mixed with this mRNA, double-stranded DNA coding for the gene of interest is produced.

•These DNA fragments are again temporarily stored in plasmid or phage libraries.

•These intronless DNA sequence codes for whatever protein the cell had been making and can be transcribed and translated by bacterial cells.



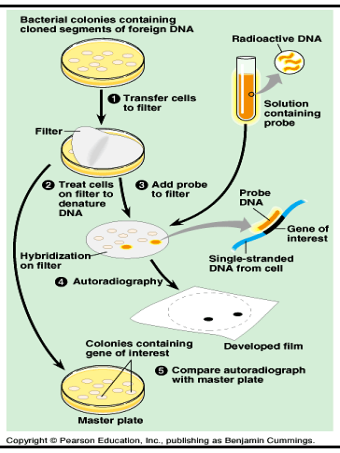
Nucleic acid probes identify clones carrying specific genes

•If some bacterial clones in the genomic library actually produce the product expressed by the gene of interest, the right clone can be isolated by testing the medium they are growing in for the product.

•If this cannot be done, scientists use radioactively labeled single-stranded nucleic acid probes, which pair with selected regions of the gene of interest.

•The cells or phages in the genomic library that hold the radioactive label are the locations of the gene in question.

•The probes can be assembled artificially if some sequence in the target protein is known.



Automation makes DNA synthesis and DNA sequencing possible

•DNA fragments can be synthesized by highly automated machines.

•Nucleotide sequences in a stretch of DNA can also be determined using automated machines. If the DNA is long, it is first cut into smaller fragments by restriction enzymes, and the fragments are sequenced separately.

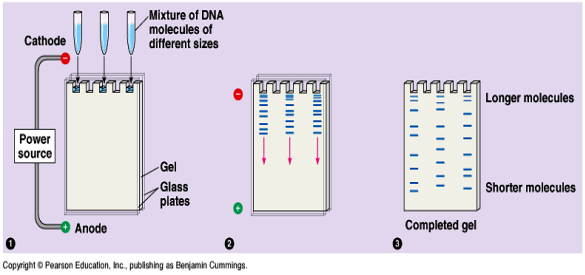
•Sequences are stored in a computer data bank and are available on a world-wide basis to scientists interested in the genes of particular organisms.

Gel electrophoresis sorts DNA molecules by size

•Gel electrophoresis sorts proteins and nucleic acids on the basis of their size and charge.

•Longer macromolecules move through the gel more slowly than do shorter macromolecules.

•The result of this differential rate of movement is a pattern of bands on the gel, each gel consisting of macromolecules of one particular size.



Restriction fragment analysis is a powerful method that detects differences in DNA sequences

•Nucleotide sequences of all but identical twins are different.

•Extracted DNA from a person's cells can be cut into a set of fragments by reacting the DNA with a series of different restriction enzymes.

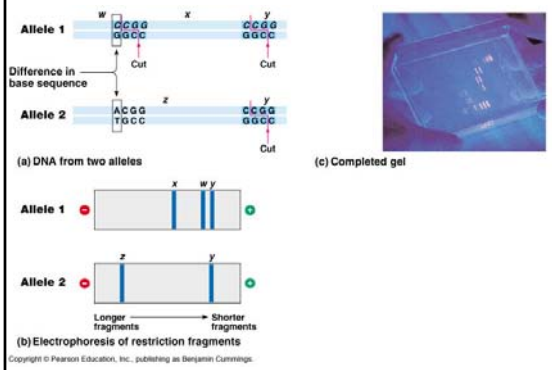
•Differences in DNA sequences on homologous chromosomes produce sets of fragments (**r**estriction **f**ragment **l**ength **p**olymorphisms, or **RFLPs**) that differ in length and number between different, nonidentical-twin individuals.

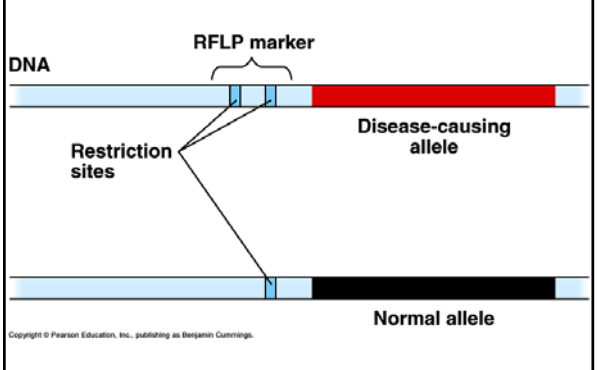
•These DNA fragments are of different length and will migrate different distances in an electrophoretic gel.

•A genetic marker is any DNA sequence whose inheritance can be tracked. It may or may not be a gene or a sequence within a gene.

•RFLP analysis was used to enable workers studying Huntington's disease to find a genetic marker closely associated with the HD gene.

•Once a genetic marker is known for a particular disease, **RFLP** analysis can be used to test for it.





The PCR method is used to amplify DNA sequences

- The **p**olymerase **c**hain **r**eaction (**PCR**) is a technique for copying a single DNA sequence many times.
- A mixture of **DNA**, **DNA polymerase**, and **nucleotide monomers** will continue to replicate, forming a geometrically increasing number of copies.
- This technique has revolutionized DNA work because sequences can now be obtained from extremely small samples.
- Prehistoric DNA from a number of sites has been cloned into partial genomes this way.