Chapter 9
Genetic Engineering

- Biotechnology: use of microbes to make a protein product
- Recombinant DNA Technology:
  - Insertion or modification of genes to produce desired proteins
- Genetic engineering: manipulation of genes/insert DNA into cells
- Gene Cloning: isolating genes from one organism, manipulating purified DNA in vitro, and transferring to another organism

It all began with

- Arber (1950)-discovered enzymes that degrade bacterial viruses
- Smith (1970)-purified the enzymes and characterized them
  - Cut DNA at specific sites
  - Called restriction enzymes

Why is genetic engineering important?

- Purify protein
  - Insulin
  - Growth factor
  - Interferon
- Generate more copies of a particular gene: “amplify DNA”
- Research gene function and regulation

TABLE 9.1 Some Pharmaceutical Products of Genetic Engineering

Product	Comments
Alpha-interferon	Therapy for leukemia, melanoma, and hepatitis; produced by *E. coli* and *Saccharomyces cerevisiae* (yeast).
Antitrypsin	Assists emphysema patients; produced by genetically modified sheep.
Beta-interferon	Treatment for multiple sclerosis; produced by mammalian cell culture.
Bone morphogenic proteins	Induces new bone formation; useful in healing fractures and reconstructive surgery; produced by mammalian cell culture.
Colony-stimulating factor (CSF)	Counteracts effects of chemotherapy; improves resistance to infectious disease such as AIDS; treatment of leukemia; produced by *E. coli* and *S. cerevisiae*.
Epidermal growth factor (EGF)	Heals wounds, burns, ulcers; produced by *E. coli*.
Erythropoietin (EPO)	Treatment of anemia; produced by mammalian cell culture.
Factor VIII	Treatment of hemophilia; improves clotting; produced by mammalian cell culture.
Gamma-interferon	Treatment of chronic granulomatous disease; produced by *E. coli*.
Hepatitis B vaccine	Produced by *S. cerevisiae* that carries hepatitis-virus gene on a plasmid.
Human growth hormone (hGH)	Corrects growth deficiencies in children; produced by *E. coli*.
Human insulin	Therapy for diabetes; better tolerated than insulin extracted from animals; produced by *Escherichia coli*.

Table 9.1.1

TABLE 9.1 Some Pharmaceutical Products of Genetic Engineering (continued)

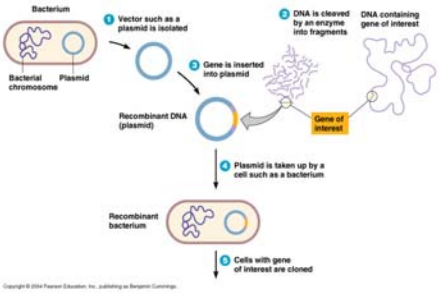
Product	Comments
Influenza vaccine	Trial vaccine made from *E. coli* or *S. cerevisiae* carrying virus genes.
Interleukins	Regulate the immune system; possible treatment for cancer; produced by *E. coli*.
Monoclonal antibodies	Possible therapy for cancer and transplant rejection; used in diagnostic tests; produced by mammalian cell culture (from fusion of cancer cell and antibody-producing cell).
Orthoclone®	Monoclonal antibody used in transplant patients to help suppress the immune system, reducing the chance of tissue rejection; produced by mouse cells.
Prourokinase	Anticoagulant; therapy for heart attacks; produced by *E. coli* and yeast.
Pulmozyme® (rhDNase)	Enzyme used to break down mucous secretions in cystic fibrosis patients; produced by mammalian cell culture.
Relaxin	Used to ease childbirth; produced by *E. coli*.
Superoxide dismutase (SOD)	Minimizes damage caused by oxygen free radicals when blood is resupplied to oxygen-deprived tissues; produced by *S. cerevisiae* and *Pichia pastoris* (yeast).
Taxol	Plant product used for treatment for ovarian cancer; produced in *E. coli*.
Tissue plasminogen activator (Activase®)	Dissolves the fibrin of blood clots; therapy for heart attacks; produced by mammalian cell culture.
Tumor necrosis factor (TNF)	Causes disintegration of tumor cells; produced by *E. coli*.

Table 9.1.2

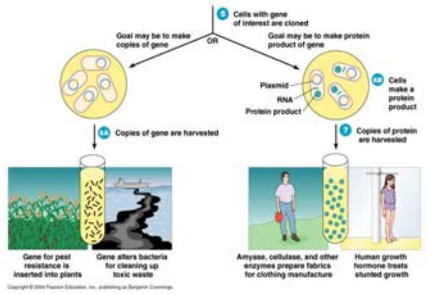
Selection

- Artificial Selection: select breeds or strains with desirable traits (eg. Antibiotic producers)
- Mutation: Mutagens cause mutations that might result in a microbe with a desirable trait
- Site-directed mutagenesis: make specific changes in gene (mutate gene so that an organism can produce more penicillin;
- 1000x more)
- Select and culture microbe with the desired mutation

The process of genetic engineering

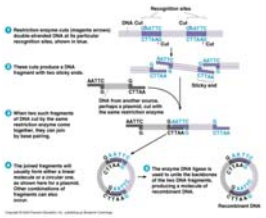


The outcomes of genetic engineering



Restriction enzymes

- Recognize a specific sequence of bases and cut the DNA backbone
- Enzymes are named from the organism they are isolated from
  - EcoR1 (GAATTC)
  - Sau3A (GATC)
- Generates “sticky ends”



Restriction Enzymes

- DNA from different organisms spliced together by binding of sticky ends
- DNA ligase then links the DNA segments

Vectors are types of DNA

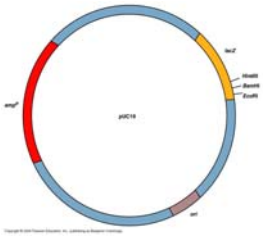
- Must be able to self replicate (WHY?)
- Must contain a promoter region
- Must a reasonable size and circular
- Often contain marker genes (antibiotic resistance genes) for easy identification of cells containing the vector

Types of Vectors

- Viral DNA
  - can carry larger pieces of foreign DNA
- Plasmids
  - pUC19 contains genes for easy selection (lacZ and amp)

Plasmids make good vectors

- pUC19 contains genes for easy selection
- Contains a polylinker region for restriction enzymes
- What happens when the plasmid is cut with EcoR1?

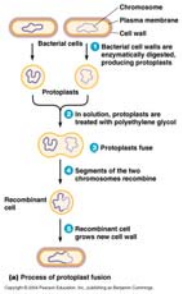


DNA can be inserted into cell by:

- Transformation
  - Naturally competent cells
  - Treat cells (E.coli, yeast, mammal cells) to make competent
    - Soak E.coli in CaCl, mix with DNA, mild heat shock

DNA can be inserted into cell by:

- Transformation
- Electroporation
  - Cells with cell wall need to be converted to protoplasts



DNA can be inserted into cell by:

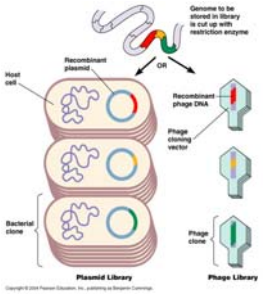
- Gene gun
  - DNA is coated with gold and propelled into the cells

DNA can be inserted into cell by:

- Microinjection
  - Glass pipette punctures the cell membrane and inserts the DNA

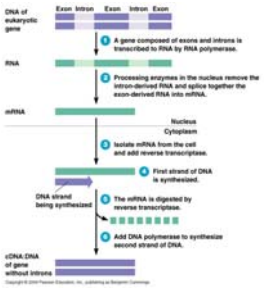
Sources of the DNA that is inserted into the vector?

- Gene library
  - collection of clones that contain every gene of an organism
  - pieces of an entire genome stored in plasmids or phages
- Synthesize DNA with a DNA machine



DNA from eukaryotic cells

- cDNA (complementary DNA)
- Problem that genes contain exons and introns
- Use reverse transcriptase synthesize cDNA from mRNA template

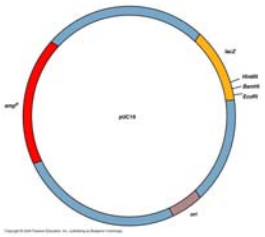


What do we have so far?

- Vectors
- Ways to get DNA into cells
- DNA of “gene of interest”
- Now we need to look at the **selection** process….how do we find the cells that have taken up the foreign DNA?

A look back at pUC19

- pUC19 has antibiotic resistance gene for anpicillin
- Also has the *LacZ* gene which codes for Beta-galactosidase
- What happens when cells take up this DNA?



Blue-White Screening

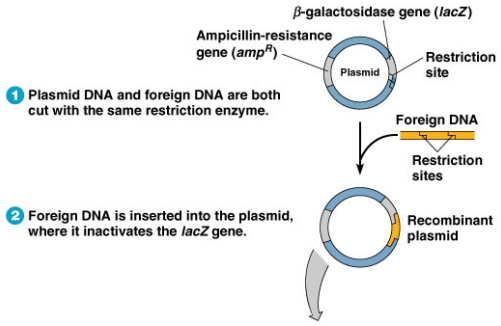


Figure 9.11.1

Blue-White Screening

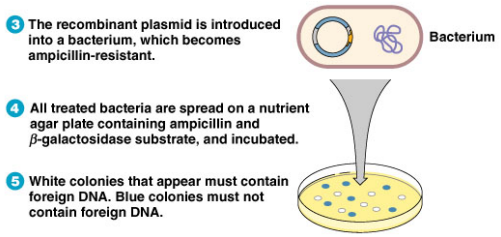
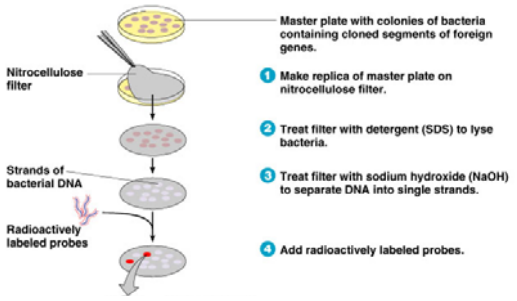


Figure 9.11.2

Colony hybridization for specific gene



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Colony Hybridization

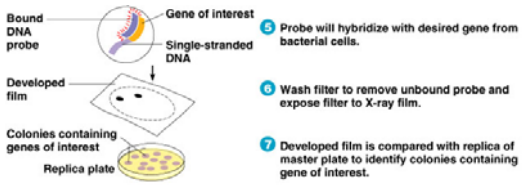
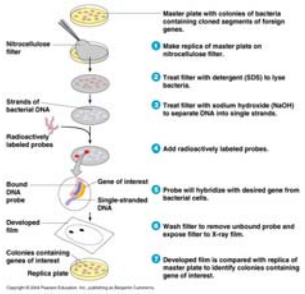


Figure 9.12.2

Colony hybridization works for finding a specific gene



E.coli

- Used because it is easily grown and its genomics are known
- Need to eliminate endotoxin from products
- Cells must be lysed to get product

DNA sequencing of a cloned piece of DNA

- Clone DNA to produce many copies to analyze sequence
- Can then be used analyze a person's DNA for the presence/absence of the gene
- Can be used to identify pathogenic strains of bacteria
- Shot gun sequencing
- Southern Blot

Random Shotgun Sequencing

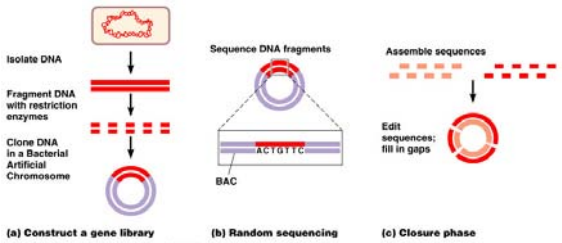
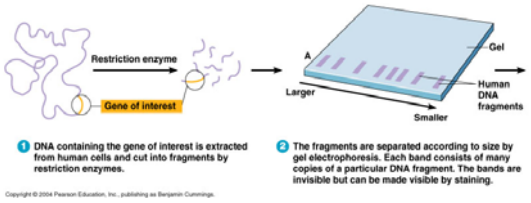


Figure 9.14

Southern Blot technique

- 1975 by Edward Southern
- Utilizes the idea of nucleic acid hybridization to target DNA
- DNA is cut into fragments with restriction enzymes
- Pieces of DNA are separated based on size on an agarose gel
- Probes are used to identify the target gene/sequence of DNA

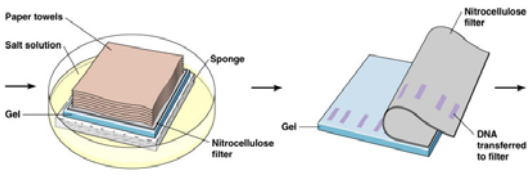
Southern Blot technique



1 DNA containing the gene of interest is extracted from human cells and cut into fragments by restriction enzymes.

2 The fragments are separated according to size by gel electrophoresis. Each band consists of many copies of a particular DNA fragment. The bands are invisible but can be made visible by staining.

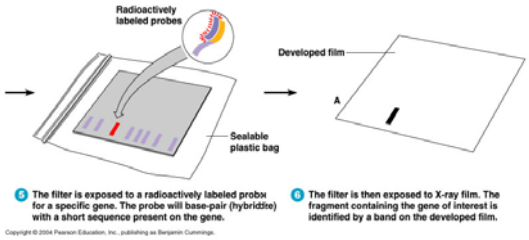
Southern Blot technique



3 The DNA bands are transferred to a nitrocellulose filter by blotting. The solution passes through the gel and filter to the paper towels.

4 This produces a nitrocellulose filter with DNA fragments positioned exactly as on the gel.

Southern Blot technique



Polymerase Chain Reaction

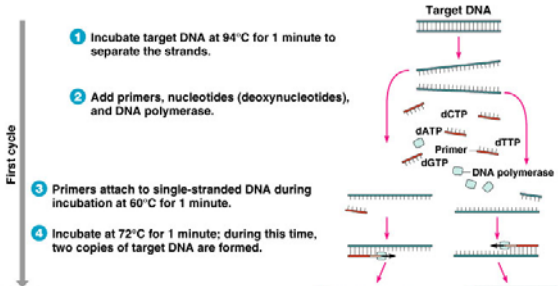


Figure 9.4.1

Polymerase Chain Reaction

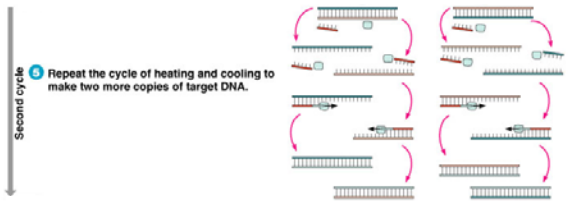


Figure 9.4.2

Polymerase Chain Reaction (PCR)

- A technique used to make more copies of DNA in vitro (enzymatically)
- Requires all the building blocks of DNA
- DNA Polymerase (Taq polymerase)
  - From thermophilic bacteria,
    Thermus aquaticus
    WHY?

Polymerase Chain Reaction (PCR)

- Used to
  - Clone DNA for recombination
  - Amplify DNA to detectable levels
  - Sequence DNA
  - Diagnose genetic disease
  - Detect pathogens

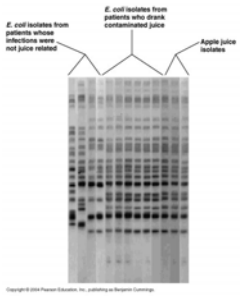
Therapeutic Applications

- Subunit vaccines
- Nonpathogenic viruses carrying genes for pathogen's antigens as vaccines
- Gene therapy to replace defective or missing genes
- Human Genome Project
  - Nucleotides have been sequenced
  - Human Proteome Project may provide diagnostics and treatments

Scientific Applications

- Understanding of DNA
- Sequencing organisms' genomes
- DNA fingerprinting for identification

DNA fingerprinting



Safety Issues and Ethics

- Avoid accidental release
- Genetically modified crops must be safe for consumption and for the environment
- Who will have access to an individual's genetic information?