Slide 1

Biotechnology

Lecture 13

Slide 2

Learning Outcomes

- Recombinant DNA
- Restriction Enzymes
- Vectors
- Clones
- Subunit Vaccines
- Polymerase Chain Reaction
- Southern Blot

Slide 3

Biotechnology and Recombinant DNA

- Biotechnology:
  - The use of microorganisms, cells, or cell components to make a product
  - Foods, antibiotics, vitamins, enzymes
- Recombinant DNA Technology:
  - Insertion of genes to produce desired proteins
  - Often inserted into bacteria like E.coli

Slide 4

Gardisil a Subunit Vaccines

- Gardasil offers women protection v HPV 6,11,16 and 18
  - Genital warts
  - Cervical Cancer
- Virus gene for HPV protein L1 moved to Yeast
- Yeast cells synthesize HPV protein
- Injected as a vaccine

Slide 5

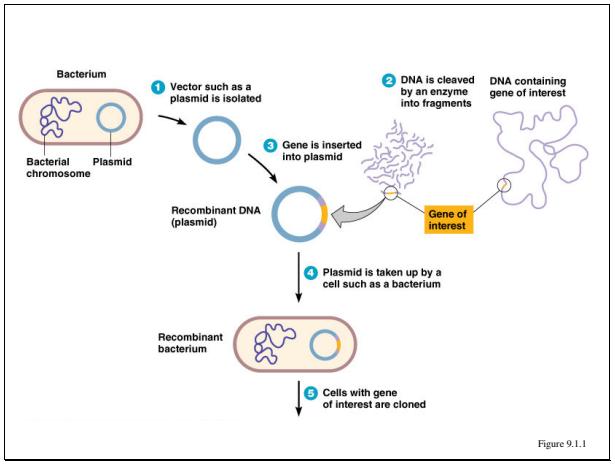


Figure 9.1.1

Slide 6

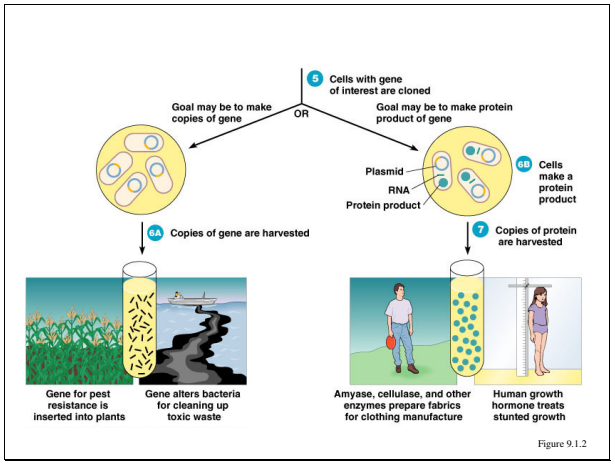


Figure 9.1.2

Slide 7

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*.

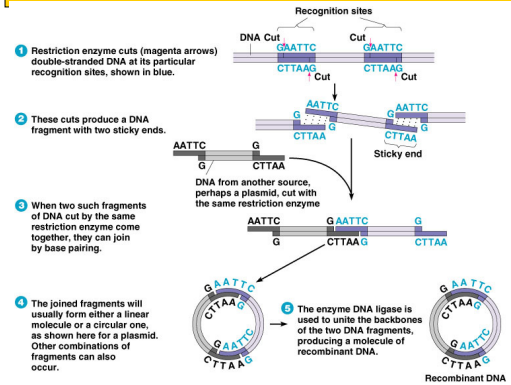
Table 9.1.1

Slide 8

Restriction Enzymes

- Cut specific sequences of DNA
- Destroy bacteriophage DNA in bacterial cells
- Hundreds of restriction enzymes now known
  - Staggered cut
  - Sticky ends – un-paired bases

Sticky Ends



DNA can be inserted into a cell by:

- Transformation
- Protoplast fusion
- Microinjection
- Electroporation
- Vectors
  - Modified viruses

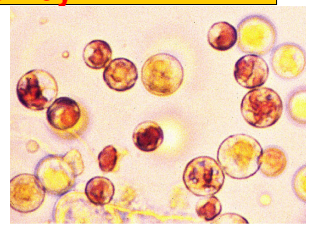


Figure 9.5b

DNA insertion

- Gene gun
- Tungsten Bullets
  - Plant cells
- Microinjection
  - Animal cells

Figure 9.6 & 7

Vectors

- Carry desired gene from one cell to cell for insertion
- Plasmids are used to insert genes into bacteria
- Genetically engineered viruses are used to insert genes into animal cells

Plasmid Vector

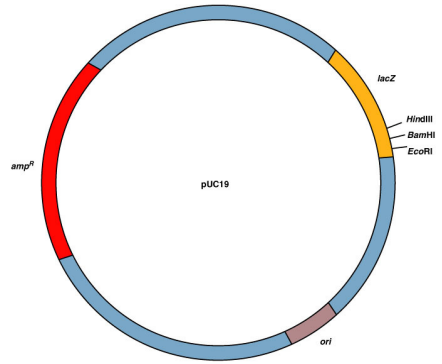


Figure 9.3

Polymerase Chain Reaction (PCR)

- To make multiple copies of a piece of DNA enzymatically
- Heat stable form of DNA Polymerase from *Thermus aquaticus*
- Used to
  - Clone DNA for recombination
  - Amplify DNA to detectable levels (CSI)
  - Sequence DNA
  - Diagnose genetic disease
  - Detect pathogens

Slide 15

Heat Stable Polymerase

First cycle

1. Incubate target DNA at 94°C for 1 minute to separate the strands.
2. Add primers, nucleotides (deoxynucleotides), and DNA polymerase.
3. Primers attach to single-stranded DNA during incubation at 60°C for 1 minute.
4. Incubate at 72°C for 1 minute; during this time, two copies of target DNA are formed.

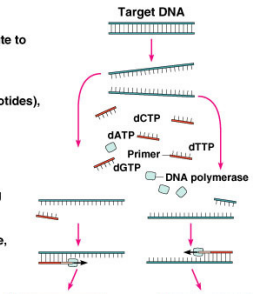


Figure 9.4.1

Slide 16

Chain Reaction

Second cycle

5. Repeat the cycle of heating and cooling to make two more copies of target DNA.

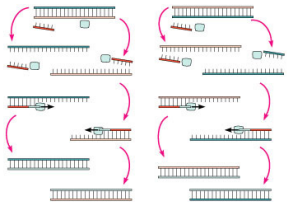
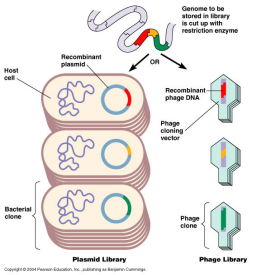


Figure 9.4.2

Gene Libraries

- Gene libraries
  - fragments of an entire genome stored in plasmids or phages
- cDNA is made from mRNA by reverse transcriptase



Genetic Engineering

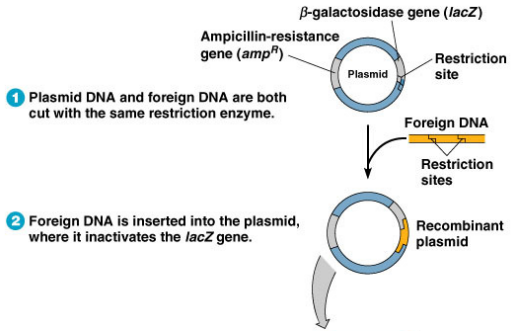


Figure 9.11.1

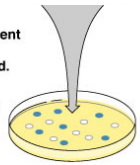
Slide 19

Genetic Engineering

3 The recombinant plasmid is introduced into a bacterium, which becomes ampicillin-resistant.

Bacterium

4 All treated bacteria are spread on a nutrient agar plate containing ampicillin and β-galactosidase substrate, and incubated.

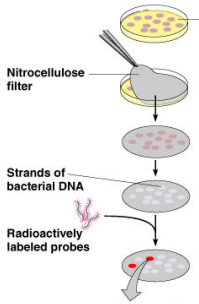


5 White colonies that appear must contain foreign DNA. Blue colonies must not contain foreign DNA.

Figure 9.11.2

Slide 20

Making a Gene Product



Master plate with colonies of bacteria containing cloned segments of foreign genes.

1 Make replica of master plate on nitrocellulose filter.

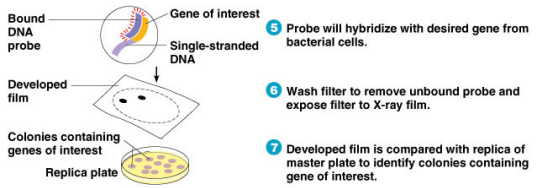
2 Treat filter with detergent (SDS) to lyse bacteria.

3 Treat filter with sodium hydroxide (NaOH) to separate DNA into single strands.

4 Add radioactively labeled probes.

Figure 9.12.1

Making a Gene Product



5 Probe will hybridize with desired gene from bacterial cells.

6 Wash filter to remove unbound probe and expose filter to X-ray film.

7 Developed film is compared with replica of master plate to identify colonies containing gene of interest.

Figure 9.12.2

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

Slide 23

Agricultural Applications

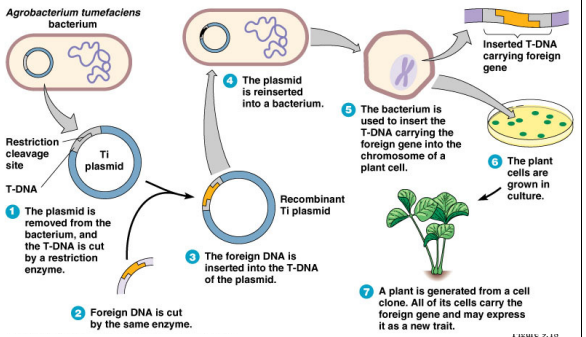
TABLE 9.2 **Some Agriculturally Important Products of Genetic Engineering**

Product	Comments
Agricultural Products	
Bt cotton and Bt corn	Plants have toxin-producing gene from *B. thuringiensis;* toxin kills insects that eat plants.
MacGregor tomatoes	Gene for pectin degradation is removed so fruits have longer shelf life.
Pseudomonas syringae, ice-minus bacterium	Lacks normal protein product that initiates undesirable ice formation on plants.
Pseudomonas fluorescens bacterium	Has toxin-producing gene from insect pathogen *Bacillus thuringiensis;* toxin kills root-eating insects that ingest bacteria.
Rhizobium meliloti bacterium	Modified for enhanced nitrogen fixation.
Round-Up® (glyphosate)-resistant crops	Plants have bacterial gene; allows use of herbicide on weeds without damaging crops.

Table 9.2

Slide 24

Genetic Engineering Using *Agrobacterium*



Safety Issues and Ethics

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