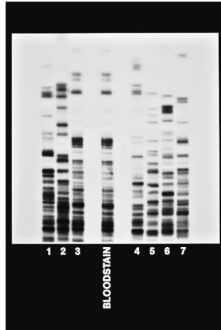


Chapter 13 Biotechnology

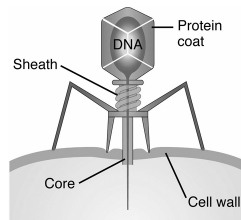


- **Biotechnology:** Commercial use of alteration of biological materials to achieve specific, applied goals.
- **Genetic Engineering:** The modification of genetic material
 - 1) Examining cellular processes (e.g. gene expression)
 - 2) Treating diseases (gene therapy)
 - 3) Generating economic / social benefits
- **Transgenic** = Organisms which express genes that have been modified / transplanted from other species.

Is This Natural?

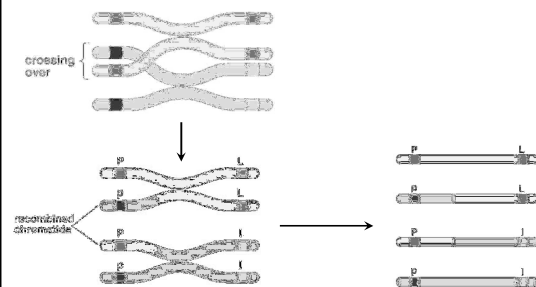
Gene modification

- **Recombinant DNA:** DNA containing genes from different organisms / species
 - ❖ Key tool in genetic engineering
- Recombinant DNA is made by exploiting natural means of recombining DNA.
 - ❖ Bacteria
 - ❖ Viruses



DNA recombination occurs in nature:

- 1) Sexual Reproduction (within species):
 - Crossing over → gametes with unique allele combinations

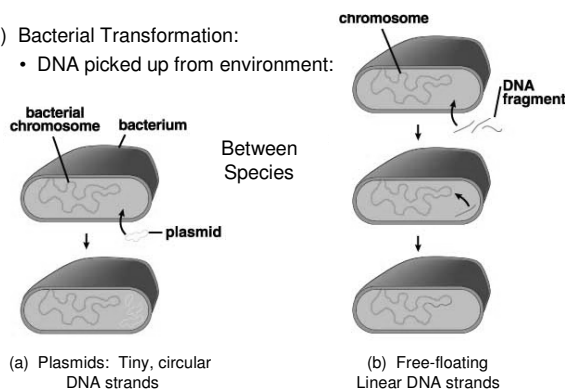


DNA recombination occurs in nature:

(Figure 13.1)

2) Bacterial Transformation:

- DNA picked up from environment:

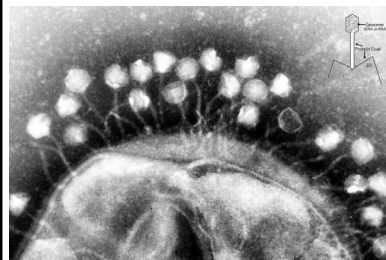


DNA recombination occurs in nature:

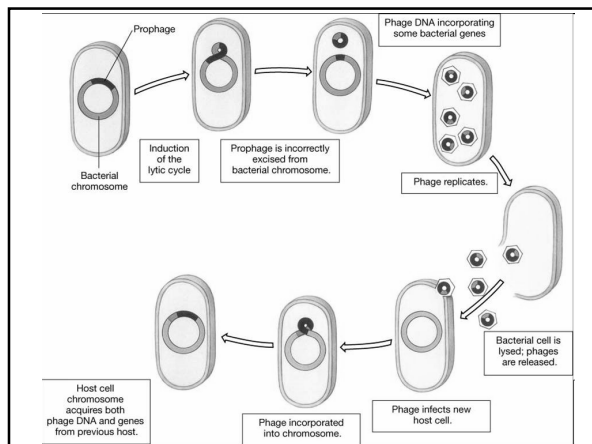
(Figure 13.2)

3) Viral Infection:

- Insertion of viral DNA into host cell / DNA
- New viruses may incorporate host genes



Viral DNA being injected into the cell by dozens of bacteriophages

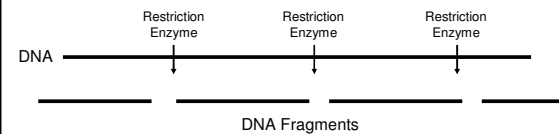


Genetic Engineering in Action:

Goal: Find functional gene in one organism and transfer gene to different organism

Step 1: Prepare Recombinant DNA

- Restriction Enzymes:
 - Cut up large DNA molecules into smaller pieces
 - Location of cut based on specific DNA sequence:

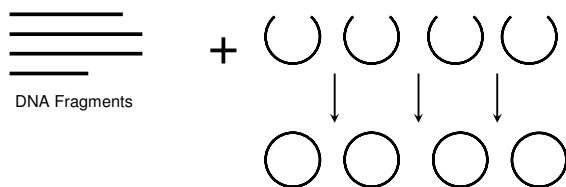


Genetic Engineering in Action:

Goal: Find functional gene in one organism and transfer gene to different organism

Step 1: Prepare Recombinant DNA

- Insert DNA fragments into vectors:
 - Vector = Specialized plasmids (bacteria) and viruses



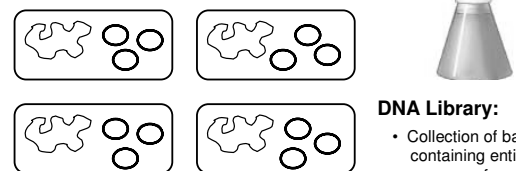
Chapter 13: Biotechnology

Genetic Engineering in Action:

Goal: Find functional gene in one organism and transfer gene to different organism

Step 1: Prepare Recombinant DNA

- Transform recombinant vectors into bacteria:
 - Bacteria randomly pick up vector



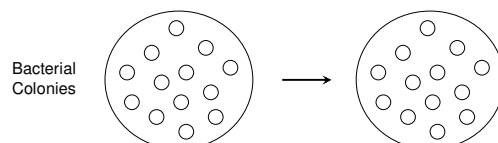
DNA Library:
• Collection of bacteria containing entire genome of organism in small pieces

Genetic Engineering in Action:

Goal: Find functional gene in one organism and transfer gene to different organism

Step 2: Find Gene(s) of Interest in DNA Library ("Clone" Gene)

- (a) Look for with DNA Probe:
- Short sequence of DNA that can form base pairs with DNA of interest



(b) Search for product of gene (protein production)

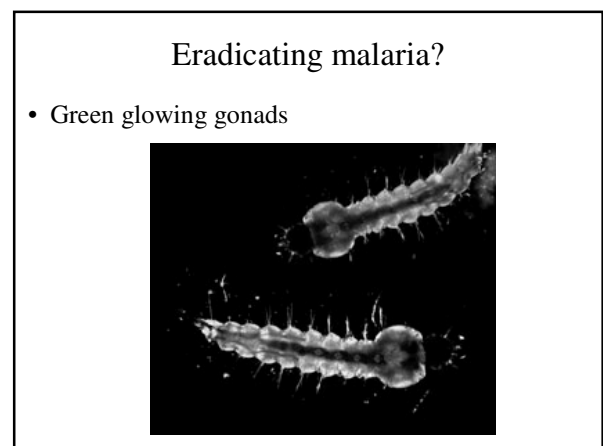
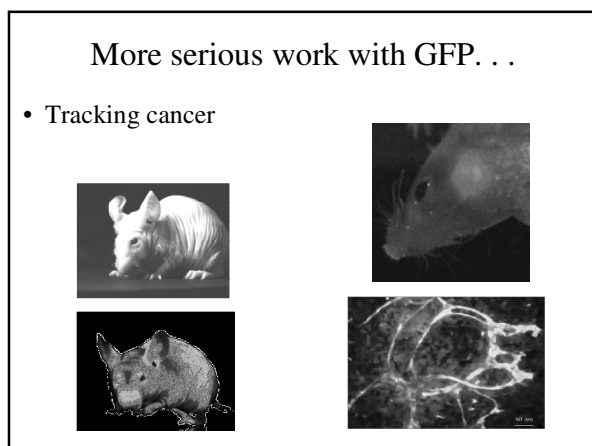
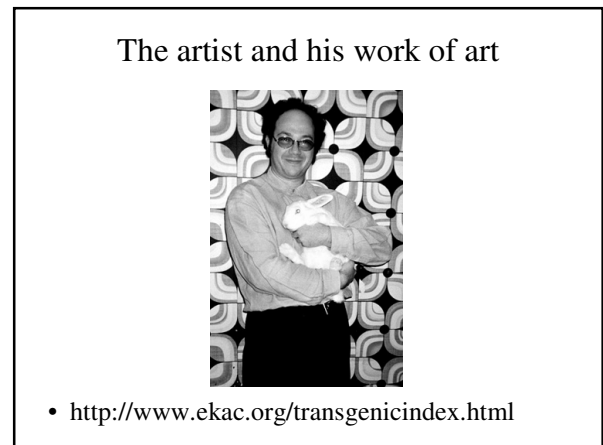
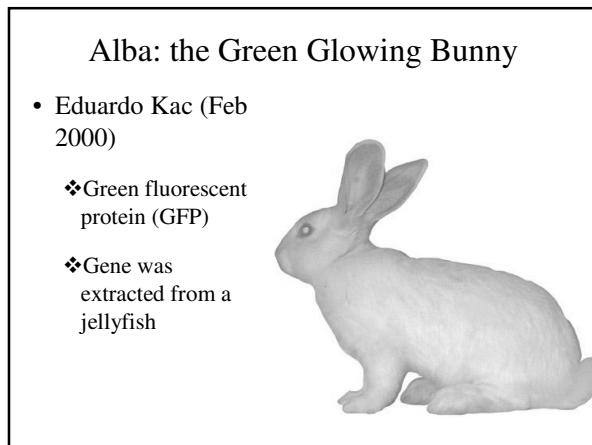
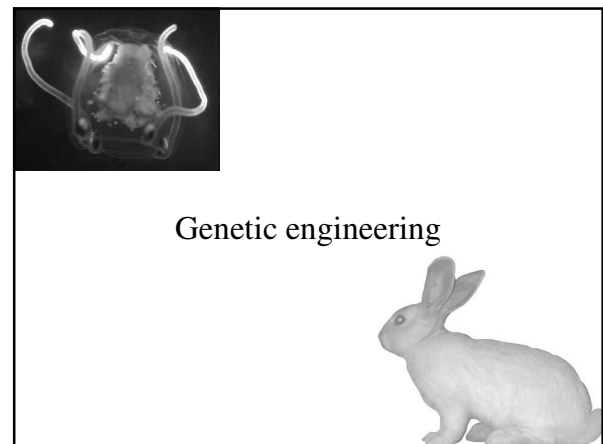
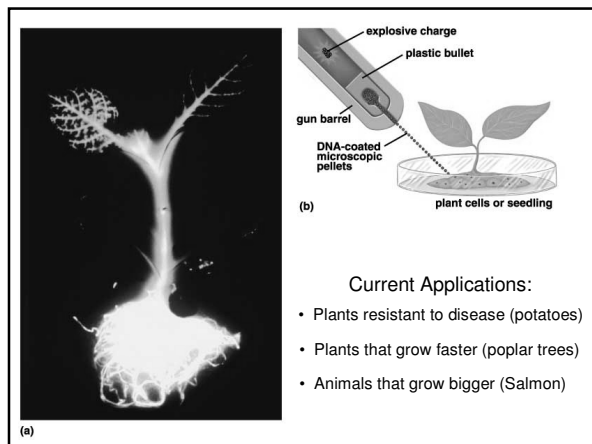
Genetic Engineering in Action:

Goal: Find functional gene in one organism and transfer gene to different organism

Step 3: Introduce gene into new organism

- Insert recombinant DNA via plasmid
- Insert recombinant DNA via virus
- Blast DNA-coated pellets into cell ("gene gun")





Knockout mice

- Knockout mice are mice where a gene is disrupted (knocked out), so it will no longer produce the protein.
- Enables researchers to determine the importance of the protein to the life of the organism.

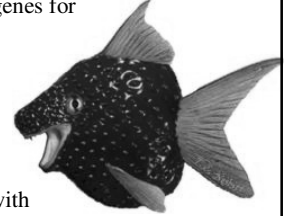


Genetically modified organisms (GMO)

- Case 1: The infamous “fishberry”
 - ❖ Strawberry with flounder genes for antifreeze proteins

❖ **NOT!**

- ❖ Experiment did not work with strawberries or tomatoes
 - Plants were not frost resistant.



What did work...

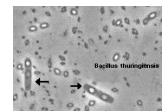
- “Frostban” 1987
 - ❖ Bacteria with one gene altered.
 - ❖ Plants sprayed with bacteria have less frost damage



First commercial genetically modified organism (GMO).

Case 2: Bt corn, butterflies and Taco Bell

- Caterpillars eat corn
- *Bacillus thuringiensis*
 - ❖ A common soil bacteria, harmless to man
 - ❖ Produces a toxin that kills caterpillars
- Monsanto developed a GM corn seed where the BT toxin gene was inserted.
 - ❖ Corn produces the toxin in every cell.
 - ❖ Caterpillars eat toxin with every bite.



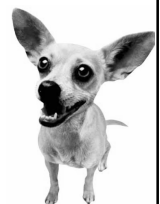
The problem

- Corn pollen ALSO has the active gene that produces toxins.
 - ❖ Monarch caterpillars eat toxin laden pollen that lands on milkweed plants.
- Controversy over the study still rages
 - ❖ Not sure of true impact of BT pollen on monarch butterflies.



Not all GMOs are OK for humans

- Genetically modified experimental corn that was not approved for human consumption accidentally was sold to Taco Bell
 - ❖ Major recall of taco shells to prevent possible health issues.
- A lot of research must take place to make sure GMO doesn't produce a toxin or allergies in humans.



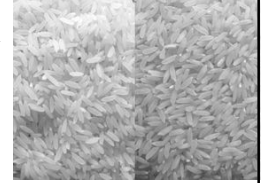
Case 3: Flavr Savr® tomatoes

- 1994: Calgene
 - ❖ Normal tomatoes cannot be shipped when ripe.
 - Tomatoes were picked green and artificially ripened by using ethylene gas.
 - ❖ A gene was inserted to make ripe tomatoes firmer
 - More survive shipment.
 - ❖ But the product was never profitable due to the high costs of development.

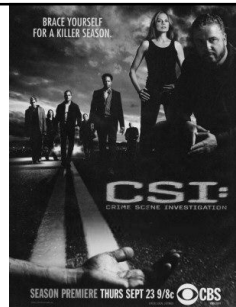


Case 4: Golden rice

- 2000
 - ❖ Modified to make Vitamin A by using a daffodil gene.
 - Care to make sure too much Vitamin A is not consumed.
 - ❖ Still not enough to make a difference
 - Milling rice removed much of the already small amount of vitamin A.

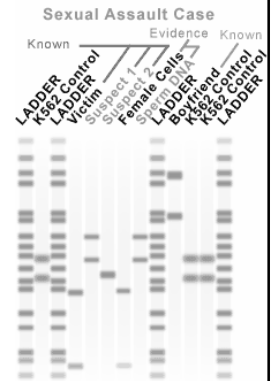


Forensic genetics



DNA Forensic analysis

- **DNA fingerprinting**
 - ❖ Originally, we used RFLPs (restriction fragment length polymorphisms).
 - ❖ PCR/VNTR (variable number tandem repeats) has replaced RFLP in modern methods
 - More likely to be unique to the individual.



1988: The very first DNA case

- 1988: Police took DNA samples from 5000 local men.

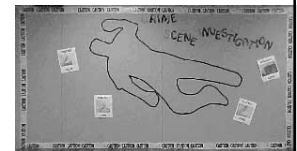


Colin Pitchfork

Pedophile that killed two young girls in England

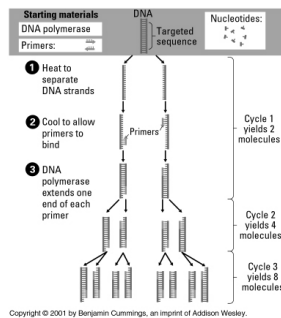
How to figure out “Who dunnit”?

- Bad guys leave traces
 - ❖ Hair with roots
 - ❖ Blood
 - ❖ Mucus
 - ❖ Semen
 - ❖ Shed skin cells
- Amplify with PCR
 - ❖ polymerase chain reaction
- <http://www.dnalc.org/ddnalc/resources/shockwave/pcranwhole.html>



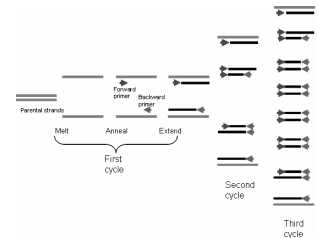
PCR requirements

- Target
- Primers
- Replication enzymes
 - ❖ DNA polymerase
 - ❖ Nitrogen rich bases



Basic Protocol

- 1. Obtain specimen
- 2. “Amplify” DNA
 - ❖ PCR: Polymerase Chain Reaction
 - ❖ How it works
 - Target DNA
 - Primers and enzyme
 - 30 cycles
 - ❖ Result
 - Lots of DNA!



Once enough DNA is made. . .

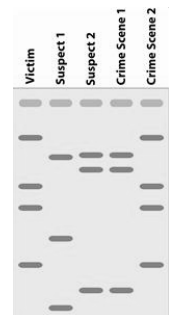
- 3. Cut with restriction enzymes
 - ❖ These enzymes are made naturally by bacteria to “chop up” virus DNA
 - ❖ Cut in very specified regions
 - CCG[^]TTG

ACCGTTGACCTCCGTTGTTATCCGTTG

↑ ↑ ↑

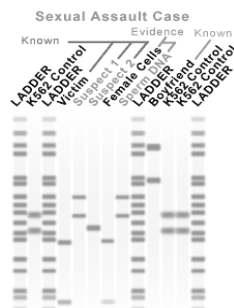
Once enough DNA is made. . .

- Cut with restriction enzymes
- Run gel
- Analyze pattern comparing victim, suspects and other involved persons.
- Crime labs now examine several different gene fragments to make a unique profile.



Create unique DNA patterns

- Human DNA : 8 billion nucleotides.
- Only rare sequences are used to make unique patterns.
- New methods create unique patterns that only occur 1 out of 20 billion people
 - ❖ Only six billion people in the world.



Case #1: the OJ trial

- Trial took place in 1994.
 - ❖ Older methods of DNA fingerprinting
 - ❖ 1 in 5 million chance of matching unique pattern.
 - ❖ Defense lawyers argued that meant that other people could have done the killing and left DNA.
 - ❖ However – LA is 3.8 million people. Likelihood is very low that another person in LA has the same pattern as OJ.
 - Even less likely that a person with the same fingerprint pattern would have known Nicole Simpson.



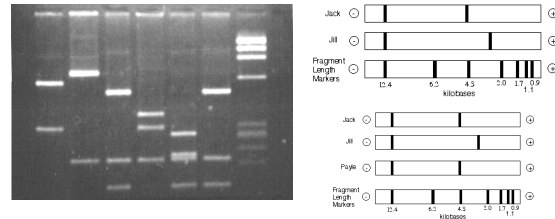
Case #2: Scott Peterson trial

- First major case that used mitochondrial DNA
 - ❖ Only transmitted by mother
 - Sperm never carries mtDNA
 - ❖ Hair found in Peterson's new boat matched mtDNA from the mother of his wife.
 - Wife supposedly never seen or was in the boat.



Case 2: Paternity analysis

- RFLP
 - ❖ Restriction fragment length polymorphism



DNA paternity testing

- Much more accurate than blood type testing
 - ❖ Many people can share the same blood type.
 - Can only remove possibility of being the father.
 - ❖ Rare DNA patterns are used that make it very unlikely another person could be the father.
 - Can indicate who is the father, unlike blood types.

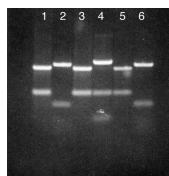
Case #1 & 2: That randy Steven Bing

- Movie producer Kirk Kerkorian
 - ❖ Married tennis star Lisa Bonder to legitimize baby.
 - ❖ Later during divorce felt that he was not the father of the child.
 - ❖ Hired detectives to search film producer Steven Bing trash for DNA.
 - Used DNA from dental floss.
 - DNA analysis revealed Bing was the father of the child.
- DNA testing also determined Bing was the father of Elizabeth Hurley's son.



Molecular Archaeology/Paleontology

- Extract DNA from ancient organisms or fossils
- Looking at sequence and patterns



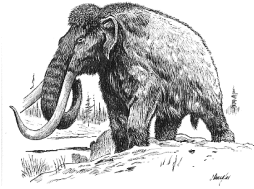
Cheddar man

- Lived ~9,000 years ago
- 23 year old man
- Killed by blow to face
- mtDNA shows relationship to several living descendants in nearby village.



Case 2: Woolly mammoth

- 40,000 years ago
- Found in permafrost
- Kazutoshi Kobayashi
 - ❖ Wants to clone!



Tasmanian wolf

- Video
- Tasmanian wolf went extinct in 1936.
- Preserved tissue still exists, and some scientists want to attempt cloning it to bring the animal back.

D. Genomics

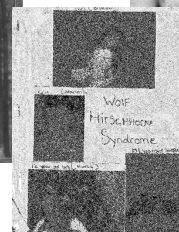
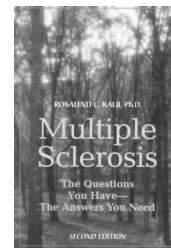
Decoding the whole DNA component

- The Human Blueprint
 - ❖ Sequencing based on 6 individuals
 - ❖ Success: April 14, 2003



Pros and cons

- Pros



Cons

