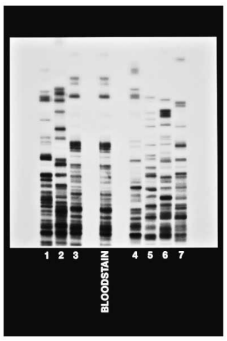
Chapter 13
Biotechnology



- **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

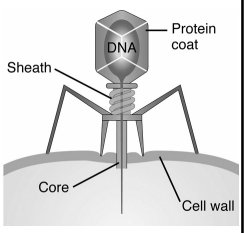
- **Transgenic** = Organisms which express genes that have been modified / transplanted from other species.

Is This Natural?

Gene modification

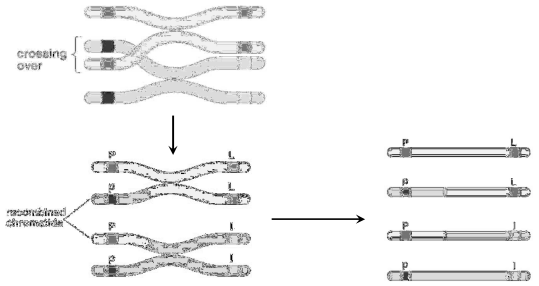
- **Recombinant DNA**: DNA containing genes from different organisms / species
  - ❖ Key tool in genetic engineering

- Recombinant DNA is made by exploiting natural means of recombining DNA.
  - ❖Bacteria
  - ❖Viruses



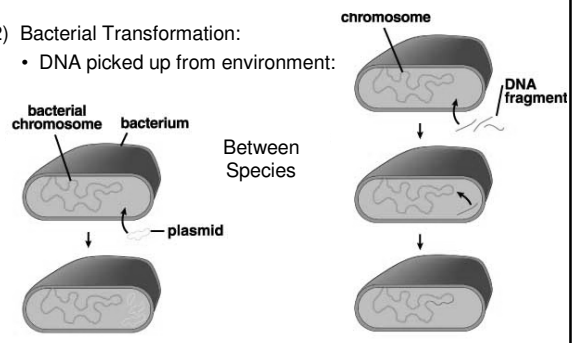
DNA recombination occurs in nature:

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:

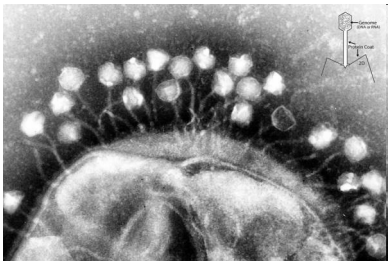


(a) Plasmids: Tiny, circular DNA strands

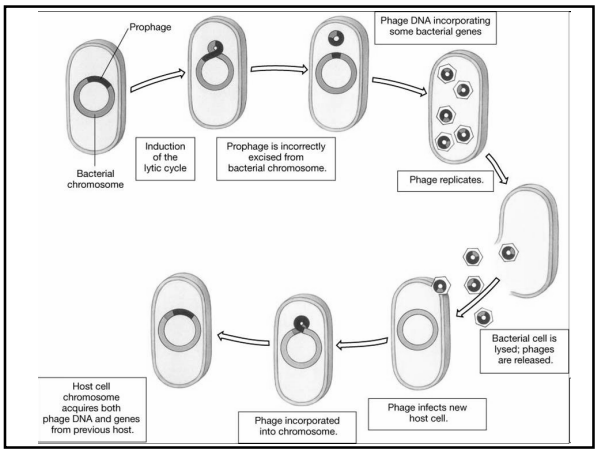
(b) Free-floating Linear DNA strands

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

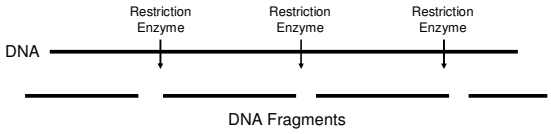


Genetic Engineering in Action:

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:

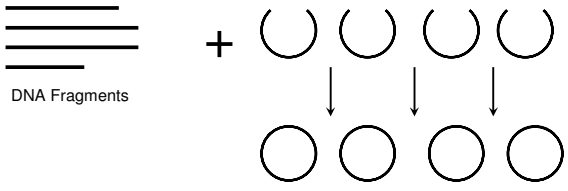


Genetic Engineering in Action:

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



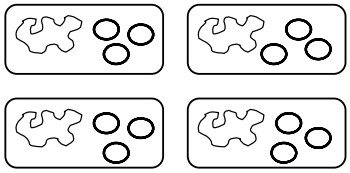
Chapter 13: Biotechnology

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:

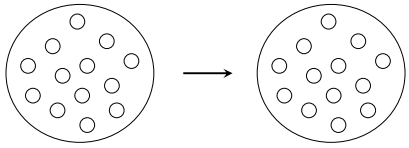
Goal: Find functional gene in one organism and transfer gene to different organism

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

Bacterial Colonies



(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

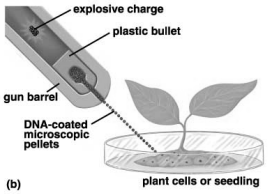
Step 3: Introduce gene into new organism

(a) Insert recombinant DNA via plasmid

(b) Insert recombinant DNA via virus

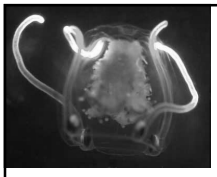
(c) Blast DNA-coated pellets into cell ('gene gun')

(a)

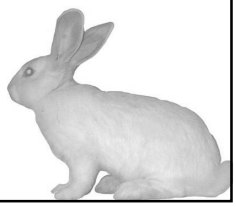


Current Applications:

- Plants resistant to disease (potatoes)
- Plants that grow faster (poplar trees)
- Animals that grow bigger (Salmon)

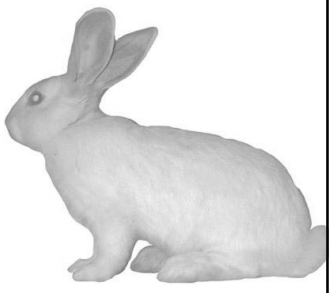


Genetic engineering



Alba: the Green Glowing Bunny

- Eduardo Kac (Feb 2000)
  - ❖Green fluorescent protein (GFP)
  - ❖Gene was extracted from a jellyfish

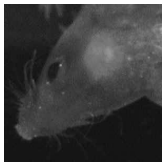


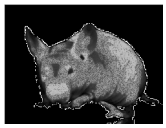
The artist and his work of art

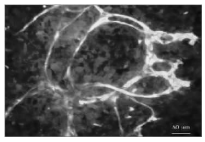
- http://www.ekac.org/transgenicindex.html

More serious work with GFP. . .

- Tracking cancer







Eradicating malaria?

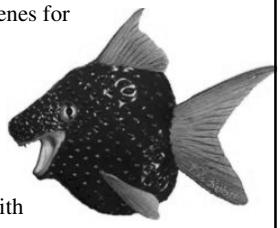
- Green glowing gonads

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.

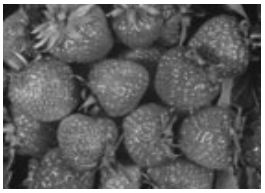
Genetically modified organisms (GMO)

- 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…

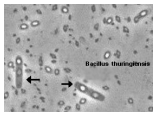
- "Frostban" 1987
  - ❖Bacteria with one gene altered.
  - ❖Plants sprayed with bacteria have less frost damage



First commercial genetically modified organism (GMO).

Case 2: Bt corn, butterflies and Taco Bell

- Caterpillars eat corn
- ***B**acillus **t**huringiensis*
  - ❖ *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

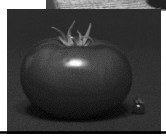
- Corn pollen ALSO has the active gene that produces toxins.
  - ❖Monarch caterpillars eat toxin laden pollen that lands on milkweed plants.
- Controversy over the study still rages
  - ❖Not sure of true impact of BT pollen on monarch butterflies.

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

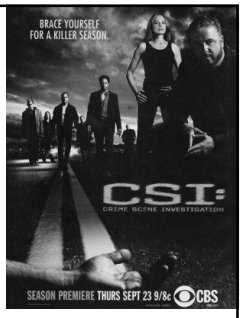
- 1994: Calgene
  - ❖Normal tomatoes cannot be shipped when ripe.
    - ➢Tomatoes were picked green and artifically ripened by using ethylene gas.
  - ❖A gene was inserted to make ripe tomatoes firmer
    - ➢More survive shipment.
  - ❖But the product was never profitable due to the high costs of development.



Case 4: Golden rice

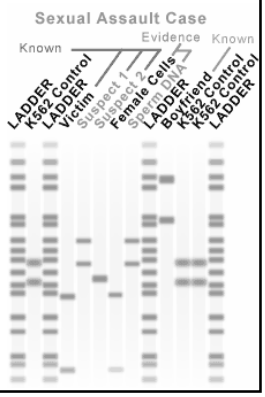
- 2000
  - ❖Modified to make Vitamin A by using a daffodil gene.
    - ➢Care to make sure too much Vitamin A is not consumed.
  - ❖Still not enough to make a difference
    - ➢Milling rice removed much of the already small amount of vitamin A.

Forensic genetics



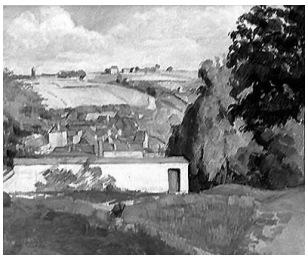
DNA Forensic analysis

- **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

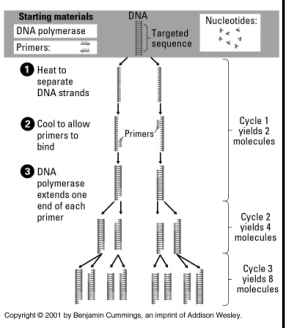
How to figure out "Who dunnit"?

- 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

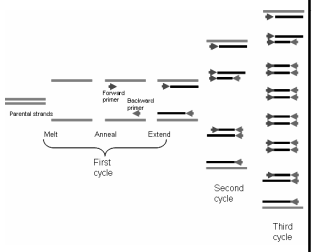
PCR requirements

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



Basic Protocol

- 1. Obtain specimen
- 2. "Amplify" DNA
  - ❖PCR: ***P***olymerase ***C***hain ***R***eaction
  - ❖How it works
    - ➢Target DNA
    - ➢Primers and enzyme
    - ➢30 cycles
  - ❖Result
    - ➢Lots of DNA!



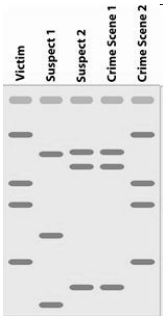
Once enough DNA is made. . .

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

A**CCGTTG**ACCT**CCGTTG**GTTAT**CCGTTG**

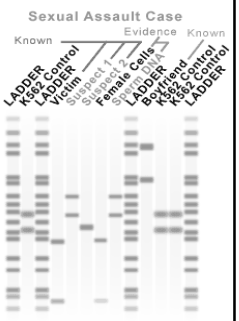
Once enough DNA is made. . .

- 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

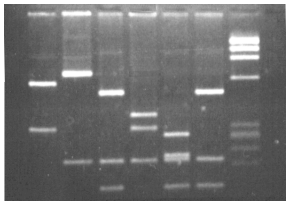
- **Trial took place in 1994.**
  - ❖Older methods of DNA fingerprinting
  - ❖1 in 5 million chance of matching unique pattern.
  - ❖Defense lawyers argued that meant that other people could have done the killing and left DNA.
  - ❖However – LA is 3.8 million people. Likelihood is very low that another 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.

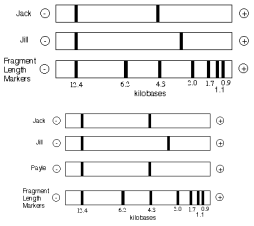
Case #2: Scott Peterson trial

- 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

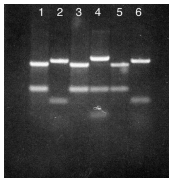
- Much more accurate than blood type testing
  - ❖Many people can share the same blood type.
    - ➢Can only remove possibility of being the father.
  - ❖Rare DNA patterns are used that make it very unlikely another person could be the father.
    - ➢Can indicate who is the father, unlike blood types.

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

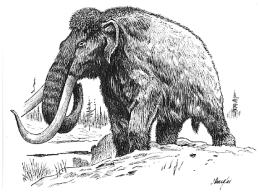


Cheddar man

- 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: Wooly mammoth

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



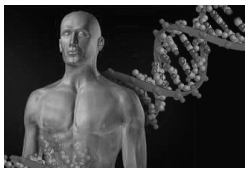
Tasmanian wolf

- 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

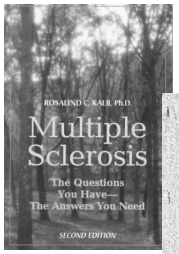
Decoding the whole DNA component

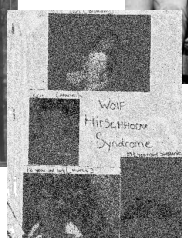
- The Human Blueprint
  - ❖Sequencing based on 6 individuals
  - ❖Success: April 14, 2003



Pros and cons

- Pros





Cons