Lecture 15
Chapter 15

Historical Developments
Basic Tools
Advanced Tools: PCR to Automated Sequencing
Applications of Molecular Biology

Historical Developments

Genetic Changes

- Humans have been changing the genetics of other species for thousands of years
  - Artificial selection of plants and animals
- Natural processes also at work
  - Mutation, crossing over

Recombinant DNA

- Paul Berg and associates were first to make recombinant DNA in 1972
- Fused fragments of DNA from one species into the genetic material from another
- Allowed them to isolate and replicate subsets of DNA from any organism

Basic Research

Recombinant DNA technology allows researchers to:

- Investigate basic genetic processes
- Reconstruct life's evolutionary history
- Devise counterattacks against rapidly mutating pathogens

DNA Sequencing

- Developed in 1977 by Maxam, Gilbert, and Sanger
- Determined the nucleotide sequence of cloned DNA fragments
- Visually rewarding, data-rich technique

The Human Genome Initiative
Goal - Map the entire human genome

- Initially thought by many to be a waste of resources
- Process accelerated when Craig Ventner used bits of cDNAs as hooks to find genes
- Sequencing was completed ahead of schedule in early 2001

Basic Tools

Discovery of Restriction Enzymes

- Hamilton Smith was studying how *Haemophilus influenzae* defend themselves from

bacteriophage attack

- Discovered bacteria have an enzyme that chops up viral DNA \
- Restriction enzymes cut DNA at a specific sequence
- Number of cuts made in DNA will depend on number of times the "target" sequence occurs

Making Recombinant DNA Using Plasmids

- Plasmid is small circle of bacterial DNA
- Foreign DNA can be inserted into plasmid
  - Forms recombinant plasmids
  - Plasmid is a cloning vector
  - Can be used to deliver DNA into another cell

Cloning Vector

- A modified plasmid that accepts foreign DNA and slips into a host bacteria, yeast, or some other cell

<u>Advanced Tools: PCR to Automated Sequencing</u>

Gene Libraries

- Bacteria that contain different cloned DNA fragments
  - Genomic library
  - cDNA library

Using a Probe to Find a Gene

- You want to find which bacteria in a library contain a specific gene
- Need a probe for that gene
  - A radioisotope-labeled piece of DNA
  - It will base-pair with the gene of interest

Amplifying DNA

- Fragments can be inserted into fast-growing microorganisms
- Polymerase chain reaction (PCR)
  - Sequence to be copied is heated
  - Primers are added and bind to ends of single strands
  - DNA polymerase uses free nucleotides to create complementary strands
  - Doubles number of copies of DNA

Primers

- Short sequences that DNA polymerase recognizes as start tags
- To carry out PCR, must first determine nucleotide sequences just before and after the gene to be copied
- Complementary primers are then created

The DNA Polymerase

- Most DNA polymerase is denatured at high temperature
- Polymerase used in PCR is from bacteria that live in hot springs

Temperature Cycles

- DNA is heated to unwind strands
- Cooled to allow base-pairing with primers and complementary strand synthesis
- DNA is heated again to unwind strands
- Cycle is repeated over and over again

DNA Fingerprints

- Unique array of DNA fragments
- Inherited from parents in Mendelian fashion
- Even full siblings can be distinguished from one another by this technique

Tandem Repeats

- Short regions of DNA that differ substantially among people
- Many sites in genome where tandem repeats occur
- Each person carries a unique combination of repeat numbers

RFLPs

- Restriction fragment length polymorphisms
- DNA from areas with tandem repeats is cut with restriction enzymes
- Because of the variation in the amount of repeated DNA, the restriction fragments vary in size
- Variation is detected by gel electrophoresis

Gel Electrophoresis

- DNA is placed at one end of a gel
- A current is applied to the gel
- DNA molecules are negatively charged and move toward positive end of gel
- Smaller molecules move faster than larger ones

Analyzing DNA Fingerprints

- DNA is stained or made visible by use of a radioactive probe

- Pattern of bands is used to:
  - Identify or rule out criminal suspects
  - Determine paternity

Genome Sequencing

- 1995 - Sequence of bacterium *Haemophilus influenzae* determined
- Automated DNA sequencing now main method
- 3.2 billion nucleotides in human genome determined in this way

Nucleotides for Sequencing

- Standard nucleotides (A,T,C, G)
- Modified versions of these nucleotides
  - Labeled so they fluoresce
  - Structurally different so that they stop DNA synthesis when they are added to a strand

Reaction Mixture

- Copies of DNA to be sequenced
- Primer
- DNA polymerase
- Standard nucleotides
- Modified nucleotides

Reactions Proceed

- Nucleotides are assembled to create complementary strands
- When a modified nucleotide is included, synthesis stops
- Result is millions of tagged copies of varying length

Recording the Sequence Genetic Engineering

- Genes are isolated, modified, and inserted into an organism
- Made possible by recombinant technology
  - Cut DNA up and recombine pieces
  - Amplify modified pieces

<u>Applications of Molecular Biology</u>

Engineered Plants

- Cotton plants that display resistance to herbicide
- Aspen plants that produce less lignin and more cellulose
- Tobacco plants that produce human proteins

- Mustard plant cells that produce biodegradable plastic

The Ti Plasmid

- Researchers replace tumor-causing genes with beneficial genes
- Plasmid transfers these genes to cultured plant cells

First Engineered Mammals

- Experimenters used mice with hormone deficiency that leads to dwarfism
- Fertilized mouse eggs were injected with gene for rat growth hormone
- Gene was integrated into mouse DNA
- Engineered mice were 1-1/2 times larger than unmodified littermates

More Mouse Modifications

- Experiments showed that human growth hormone genes can be expressed in mice
- Human genes are inserted into mice to study molecular basis of genetic disorders, such as Alzheimer's disease
- Variety of methods used to introduce genes

Designer Cattle

- Genetically identical cattle embryos can be grown in culture
- Embryos can be genetically modified
  - Experimenters are attempting to create resistance to mad cow disease
  - Others are attempting to engineer cattle to produce human serum albumin for medical use

Where Do We Go Now?

- Can we bring about beneficial changes without harming ourselves or the environment?
- Gene therapy is not harmless
  - A young man died after gene therapy that used an adenovirus
- Gene therapy can save lives
  - Infants with disabled immune systems are now healthy

Eugenic Engineering

- Selecting "desirable" human traits
- Who decides what is desirable?
- 40 percent of Americans say gene therapy to make a child smarter or better looking would be OK

Xenotransplantation

- Transferring an organ from one species into another
- Researchers have succeeded in “knocking out” a gene in pigs so that transplantation of their organs might not be identified and rejected by the human immune system
- However, such transplantation could invite viruses to infect humans in a catastrophic manner

Effects of Engineered Organisms

- Opposition to any modified organisms
- What if engineered genes escape into other species?

Genomics

- Research field that investigates genomes of humans and other species

Using Human Genes

- Even with gene in hand it is difficult to manipulate it to advantage
- Viruses usually used to insert genes into cultured human cells but procedure has problems
- Very difficult to get modified genes to work where they should

DNA Chips

- Microarrays of thousands of gene sequences representing an entire genome – all stamped onto a glass plate about the size of a business card