Ch. 9: Frontiers of Biotechnology
9.1: Manipulating DNA

Restriction Enzymes

- AKA endonucleases
- Cut DNA at precise locations (restriction sites) based on nucleotide sequence
- Recognize sequences 4 – 8 base pairs long
- Obtained from bacteria
  - Used by bacteria to restrict effect of a virus
9.1: Manipulating DNA

Two types of Restriction Enzyme:

- Yield different types of cuts:
  - Blunt ends: straight cut
  - Sticky ends: staggered cut
  - Often used in biotech
  - Complimentary BP’s will hydrogen bond
9.1: Manipulating DNA

Sticky Ends
9.1: Manipulating DNA

Once cut, DNA sequence can be studied

- e.g. gene sequencing
- e.g. gene can be cut out from DNA and placed into the DNA of another organism
9.1: Manipulating DNA

Gel Electrophoresis *(Fig. 9.3, p. 267)*

- Method used to separate DNA fragments by size (length)
  - DNA is negatively charged
  - Place DNA sample on gel and pass electricity through gel
  - DNA migrates toward positive electrode
  - Smaller fragments move faster (farther)
  - Fragments appear as bands (lines) on the gel, forming a restriction map.
9.1: Manipulating DNA

Gel Electrophoresis *(Fig. 9.3, p. 267)*

- Can be used to diagnose genetic disease by comparing length of fragment with normal DNA
9.2: Copying DNA

- Amt. of DNA from individual sample is insufficient for most studies, so . . .

Polymerase Chain Reaction (PCR)

- Process that produces multiple (millions or billions) copies of DNA sequence rapidly

- Developed by Kary Mullis
9.2: Copying DNA

1. **Separating:** Heat DNA (90°C/194°F) to separate the two strands
   a. Also breaks down DNA polymerases, so . . .
   b. Use polymerases from heat tolerant organism, *Thermus aquaticus*, found in Yellowstone hot springs: **Taq polymerase**

   - Note: Patent for PCR process sold for $300 million. NPS never received any royalties
2. **Binding:** Cool DNA (55°C/131°F) so primer can bind to each DNA strand.
9.2: Copying DNA

3. Copying: Heat sample again (72°C/152°F)
   a. DNA polymerases build new complimentary strands of DNA
9.2: Copying DNA

- Run cycle 30 times $\rightarrow$ over 1 billion copies
  - Makes 1 million copies/hr
9.3: DNA Fingerprinting

DNA Fingerprint: a representation of parts of an individual’s DNA
- A specific type of restriction map
- Used to identify a person at the molecular level
9.3: DNA Fingerprinting

DNA Fingerprint:

- One’s DNA fingerprint is a combination of one’s parents’ fingerprints (Fig. 9.6, p. 272)

- Greatest differences are in noncoding sections of DNA – *repeating sequences*
  - Number of repeats differs from one person to another
9.3: DNA Fingerprinting

DNA Fingerprinting Method:

1. Cut DNA in known locations with restriction enzymes
2. Separate fragments with gel electrophoresis
3. The more repeats $\rightarrow$ the larger the DNA fragment
   - Unlikely more than one individual will have multiple fragments in common – even among brothers and sisters
   - Usually compare five (5) regions of the genome
9.3: DNA Fingerprinting

**Probability**

**Suppose:**
- 1 in 500 people have matching DNA in region A,
- 1 in 90 have matching DNA in region B
- 1 in 120 have matching DNA in region C

**What is the chance that two people will match in all three regions?**

\[
\frac{1}{500} \times \frac{1}{90} \times \frac{1}{120} = 0.000000185
\]

1 chance in 5.4 million people!
9.3: DNA Fingerprinting

Uses:
- To determine guilt or innocence
  - Easier to prove innocence than guilt
    - Possible contamination of sample
    - No legal standard for probability of random match
- To determine family relationships
- To study biodiversity
  - e.g. ID native Galapagos tortoises or locate genetically engineered crops
9.4: Genetic Engineering

Examples:
- Glowing plants used to track genetically modified crops
- Glowing mice used in cancer research
- Glowing yeast used to locate water pollution
9.4: Genetic Engineering

Clone: a genetically identical copy of a single gene or entire organism
- Asexual reproduction in plants
- Bacterial reproduction
- Identical twins
- Regeneration in sea stars
9.4: Genetic Engineering

To clone a mammal:

Swap DNA between cells (nuclear transfer)

1. Obtain unfertilized egg and remove nucleus
2. Implant nucleus from cell of animal to be cloned
3. Stimulate egg to begin cell division
4. Transplant embryo into female

Cows, pigs, mice, your pet!!!
9.4: Genetic Engineering

- Clone may not look like or act like the original.
- Environment plays a role in gene expression.
9.4: Genetic Engineering

Uses:
- Clone organs from mammals for transplant into humans
- Save endangered species

Concerns:
- Health of clone
  - Dolly aged quickly – possibly from having “old” DNA
- Ecological biodiversity reduced
  - Population more susceptible to disease, etc.
9.4: Genetic Engineering

Genetic Engineering: changing an organism’s DNA to give it new traits

- Uses DNA with genes from more than one organism (recombinant DNA)

Possible uses:
- Produce crops that make medicines or vitamins ("pharming")
- Make vaccines that protect against HIV
9.4: Genetic Engineering

Genetic Engineering:

- Bacterial **plasmids** used to manufacture large amounts of targeted gene
- See Fig. 9.11, p. 277 for procedure

Bacteria become “gene factories”

Bacteria are **transgenic** – an organism that has one or more genes from another organism
9.4: Genetic Engineering

Genetic Engineering in Plants:

- Allow transgenic bacteria to infect plant → new gene becomes part of plant’s DNA
- Genetically modified plants (GM plants)
- Scientists can give plants new traits, e.g.
  - Resistance to frost, insects, disease
  - *Bt* gene = a natural pesticide
  - Modify to increase crop yield, e.g. potatoes and corn
9.4: Genetic Engineering

Genetic Engineering in Animals:

- More difficult

  - Animals are more resistant to genetic modification
    - Only small percentage mature normally
    - Of those that do, only a few develop into a transgenic animal
9.4: Genetic Engineering

Genetic Engineering in Animals:

Process:

1. Obtain fertilized egg cell
2. Insert foreign DNA into nucleus
3. Implant egg back into female
9.4: Genetic Engineering

Genetic Engineering in Animals:

- **Uses:**
  - Transgenic mice to study cancer and cancer drugs (*oncomouse*), diabetes, brain function & development, sex determination.
  - **Gene knockout:** genes purposely “turned off” in mice to study gene function, genetic diseases, obesity (gene for leptin, which controls hunger).
9.4: Genetic Engineering

Concerns:
- Insufficient research done so far?
- Possible side-effects of GE organisms
  - Allergic reactions?
  - Environmental decrease in genetic diversity
9.5: Genomics and Bioinformatics

Genomics: study of genomes

- **Genome**: sequence of an organism’s genetic information, for one gene or for all its DNA

Comparing DNA of many different people can:

- Find genes that cause disease
- Understand how medicines work in different individuals
- Learn evolutionary relationships
- Learn how genes interact
- Determine how one’s genome makes that individual unique
Comparing DNA of many different people can:

- Find genes that cause disease
- Understand how medicines work in different individuals, e.g. an organism’s DNA is used as a model in medical research
- Learn evolutionary relationships
- Learn how genes interact
- Determine how one’s genome makes that individual unique
9.5: Genomics and Bioinformatics

**Gene sequencing:** Determining the order of DNA nucleotides in genes or entire genomes

- Frederic Sanger (1970’s) developed technique
- See Fig. 9.13, p. 281 for comparison of selected genomes
9.5: Genomics and Bioinformatics

Human Genome Project:

- **Goals:**
  - Map and sequence all of DNA base pairs of all 24 human chromosomes (finished in 2003)
  - Identify all of the genes within the sequence

- **Current work:**
  - Identifying genes
  - Locating genes on chromosomes
  - Determining gene function
9.5: Genomics and Bioinformatics

Bioinformatics: Use of computer databases to organize and analyze biological data

- Store, share, find data
- Compare genomes of various individuals or organisms
- Predict and model gene and protein function
DNA Microarrays: Tools for studying many genes at once

Small chips dotted with multiple genes in a grid pattern
DNA Microarrays:

1. Make fluorescently labeled single-stranded cDNA from mRNA
2. Add labeled cDNA to microarray
3. cDNA binds to genes in array; gene glows
4. Glowing indicates which genes were expressed (mRNA was synthesized) in that cell

- Can compare gene expression in cancer vs. healthy cells, for instance
9.5: Genomics and Bioinformatics

**Proteomics:** Comparison and study of all proteins resulting from one’s genome.

- Studies protein function and interaction
- More difficult than studying genes
  - A single gene can code for >1 polypeptide
  - Different cells/tissues have different proteins
  - Protein function must often be studied in living, functional organism
9.5: Genomics and Bioinformatics

Potential benefits:

- Study evolutionary relatedness (phylogenies)
- Learn how proteins are involved in disease
  - Cancer, heart disease, arthritis
  - Develop new treatments that target proteins involved
  - May be able to match medical treatment to individual patient, based on their unique body chemistry
9.6: Genetic Screening & Gene Therapy

**Genetic Screening:** Process of testing DNA to determine a person’s risk of having, or passing on, a genetic disorder

- Screens for specific genes known to be associated with certain disorders, e.g. certain cancers (e.g. BRCA1 gene – breast cancer), cystic fibrosis, Duchenne’s muscular dystrophy
- Not all defective genes have been identified yet
9.6: Genetic Screening & Gene Therapy

Genetic Screening:
- Can save lives
- Can \(\rightarrow\) difficult choices
  - How would knowledge you had a gene for a terminal illness affect your daily living?

Ethical dilemmas:
- Should screening be mandatory?
- Should employers/health insurance providers have access to this information?
9.6: Genetic Screening & Gene Therapy

**Gene Therapy:** Replacement of a defective or missing gene; addition of a new gene

- Still largely experimental
- Must get new gene into proper cells
- Cells must incorporate new gene into their DNA
9.6: Genetic Screening & Gene Therapy

One method:

1. Obtain sample of bone marrow stem cells
2. "Infect" cells with GE virus with new gene
3. Replace stem cells in person’s bone marrow
4. Stem cells divide and make more blood cells with the new gene
9.6: Genetic Screening & Gene Therapy

1. Cells are removed from patient.
2. In the laboratory, a virus is altered so that it cannot reproduce.
3. A gene is inserted into the virus.
4. The altered virus is mixed with cells from the patient.
5. The cells from the patient become genetically altered.
6. The altered cells are injected into the patient.
7. The genetically altered cells produce the desired protein or hormone.
9.6: Genetic Screening & Gene Therapy

1990: First successful trial

- Two children with genetic autoimmune disorder → Now living healthy adult lives
- “Suicide” genes may be inserted into cancer cells → Kill only cancer cells
9.6: Genetic Screening & Gene Therapy

Challenges:
- Must get correct gene into correct cells
- Gene expression must be regulated
  - Avoid too much or too little of the gene’s protein
- Will new gene affect other gene’s activities?
- So far, few positive long-term results