

Chapter 20

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- Genes can be inserted into animals and make them glow
- The Human Genome has 3.2 billion bases
- Genetic Engineering
 1. Manipulation of DNA
- Bacteria
 1. One-celled prokaryotes
 2. Reproduce by mitosis
 3. Rapid growth (generation every 20 minutes)
 4. Dominant form of life on Earth
- Bacterial Genome
 1. Single, circular chromosome
 2. Naked DNA (no histone proteins)
 3. Contain $\frac{1}{1000}$ of the DNA of a eukaryote
- Plasmids
 1. Small, supplemental circles of DNA
 2. Carry extra genes (2-30 genes, usually for antibiotic resistance)
 3. Can be exchanged between bacteria (rapid evolution)
 4. Can be taken from the environment
- Plasmids allow for an easy way to insert genes into a bacteria
 1. Insert new gene into plasmid

2. Insert plasmid into bacteria
 3. Bacteria now expresses new gene (bacteria make new protein)
- Example: Insulin can be farmed from bacteria by inserting the insulin gene into a plasmid, and waiting for bacteria to reproduce
 - DNA is cut using restriction enzymes
 1. Evolved in bacteria to cut up foreign DNA
 2. “Restrict” the action of the attacking organism
 3. Protects against viruses and other bacteria
 4. Bacteria protect their own DNA by not using the base sequences recognized by the enzymes in their own DNA
 - Restriction Enzymes
 1. Cut DNA at specific sequences (palindromes)
 2. Produces protruding ends (sticky ends — will bind to any complementary DNA)
 3. Many different enzymes
 - The same insulin in bacteria can be used in humans because the “code” is universal
 - Transformation
 1. Alteration of a bacterial cell’s genotype and phenotype by the uptake of foreign DNA from the surrounding environment
 2. Insert recombinant plasmid into bacteria
 3. Grow recombinant bacteria in agar cultures — bacteria make lots of copies of plasmids (plasmid “cloning”)
 4. Production of many copies of inserted gene
 5. Production of “new” protein
 - Bacteria Lab:
 1. Normally, *E.coli* does not grow when ampicillin is around
 2. Insert a new gene into *E.coli* to make it resistant to the antibiotic ampicillin
 - Genetically modified organisms (GMOs) are also a product of biotechnology
 1. Enabling plants to produce new proteins
 2. Protect crops from insects — BT corn
 - (a) Corn produces a bacterial toxin that kills corn borer (caterpillar pest of corn)

3. Extend growing season — fishberries
 - (a) Strawberries with an anti-freezing gene from flounder
- How do we compare DNA fragments?
 1. Separate fragments by size
 2. Run it through a gelatin (agarose gel)
 3. Gel electrophoresis
 - Gel Electrophoresis
 1. A method of separating DNA in a gelatin-like material using an electric field
 2. DNA is negatively charged
 3. DNA moves to the positive side
 - DNA moves in an electrical field — size of fragments affects how far it travels (small pieces travel farther, large pieces travel slower and lag behind)
 - Gel Electrophoresis Uses:
 1. Useful for comparing DNA samples from different organisms to measure evolutionary relationships
 2. Useful in medical diagnosis (e.g. Huntington's disease)
 3. Useful in forensics, such as comparing the DNA sample from a crime scene with that of suspects and victim
 4. Useful for comparing blood samples to determine who blood belongs to (DNA fingerprinting) by comparing DNA banding
 5. Useful for determining paternity — the more bands shared with a person, the more likely they are a parent
 - Differences at the DNA level
 1. Sections of “junk” DNA
 - (a) Doesn't code for proteins
 - (b) Made up of repeated patterns
 - i. CAT, GCC, and others
 - ii. Each person may have a different number of repeats
 - (c) Many sites on our 23 chromosomes with different repeat patterns
 - PCR — Polymerase Chain Reaction
 1. Method for making many, many copies of a specific segment of DNA

2. Only need 1 cell of DNA to start

- PCR Process

1. DNA replication in a test tube — template strand, DNA polymerase enzyme, nucleotides (ATP & GTP), and primers are necessary
2. Primers are critical — a bit of the sequence needs to be known to make proper primers
3. Primers bracket target sequence
 - (a) Start with a long piece of DNA and copy a specified shorter segment
 - (b) Primers define section of DNA to be cloned
4. Process Steps:
 - (a) In tube: DNA, DNA Polymerase Enzyme, Primer, and Nucleotides
 - (b) Denature DNA: heat (to around 90[° C]) DNA to separate strands
 - (c) Anneal DNA: cool to hybridize with primers and build DNA (extension)