Unit 4: Molecular Genetics
Content Outline: DNA Biotechnology (4.4) - Part 1

I. **Genetic Engineering** (The field of science dealing with *manipulating* genomes.)
- A. **Recombinant DNA** is the major focus of genetic engineering.
  1. In this process, DNA from two different sources is joined into *one* molecule.
- **B. Biotechnology** (This term refers to the use of computers and other devices to help in performing science.)
  1. DNA gene cloning is an example.
     a. This process involves the bacterial **plasmids** and another DNA source.
     b. A **plasmid** is a small ring of DNA found in bacteria *in addition* to the main large circular DNA strand found in the nucleoid region. (Please make sure students understand the difference between the two DNA strands.)

II. **Bacterial Cloning** Process. This is used for *inserting single genes* into bacteria.
- A. Step 1: **Restriction Enzymes** are used to cut a DNA plasmid and DNA from other donor source.
  1. Restriction enzymes cut DNA at *specific nucleotide sequences*.
     The specific DNA sequence is referred to as the **Restriction Site**.
  2. Genes of interest (and antibiotic resistance genes) are cut into **Restriction Fragments.**
  3. When the DNA is cut, "Sticky Ends", or single-stranded DNA ends are created.
  4. *The same **restriction enzyme** must be used on both the plasmid and the DNA donor source.*
     a. Therefore, the "sticky ends" of the plasmid and the genes of interest *will match and can be joined.*
- B. Step 2: Create conditions for bacteria to take up *recombined* plasmids (bacteria are now **transformed**).
- C. Step 5: Incubate transformed bacteria in the presence of antibiotic to select for *only* transformed cells. (Students may need help understanding the difference between the transformed and non-transformed being on the same plate. An easy quick demo would be to take a paper bag and dump in folded pieces of paper to it. Make sure to have enough to allow all students to draw one piece. On some (about 5-10) put the word transformed on it. Then have those students with "transform" stand up. They survived the others did not.)
- D. Step 5: *Transformed* bacteria reproduce by binary fission to achieve a *large working population*.
- *E. Outcome: Transformed cells (that have antibiotic resistance) can express the gene of interest.*

III. **Polymerase Chain Reaction (PCR)** – This process requires *no organism* in the production of new DNA molecules.
- A. Kary Mullis won a Nobel Prize in 1993 for the development of this process.
  1. The process is used to turn a single molecule of DNA into a *large, workable sample of 100% identical DNA molecules*. This is widely used in criminal forensics (Murder cases).
  2. Nobel Prize is the *top award* a scientist could receive for their research. It would be like an MVP award in sports or an Oscar for actors or a Grammy for singers.
- B. Process
  1. The DNA sample is placed in a **PCR Thermal Cycler** machine.
     a. The machine uses *heat, DNA Primers, enzymes and a constant supply of nucleosides* to build *new* DNA molecules that are *identical* in nucleotide sequence to the original molecule.
     b. First step: *Heat* is used to *separate the DNA double helix* so that replication can occur.
     c. Second step: The *attachment of a DNA Primer* to the template DNA strand will start replication.
     d. Third step: The *DNA polymerase enzyme works 5'→3'* attaching nucleosides to the growing "new" side of the replicated DNA molecule.
     e. Fourth step: *Cool* the mixture to recombine and stabilize the DNA back into a double strand.
     f. *Repeat the cycle many more times* to get large, workable sample of the DNA.
     g. Perform a Gel Electrophoresis test to separate for comparison of nucleotide sequences between organisms.

Part 2

I. Gel Electrophoresis

A. This process is used to create a "*DNA fingerprint*".
(good place to remind students that fingerprints are unique to each person. No one has the exact same *set* of fingerprints, even identical twins. So we can use them to identify people, such as criminals.)

B. *Different DNA samples* are exposed to the *same* restriction enzyme.
   1. This creates **Restriction Fragment Length Polymorphisms (RFLP's)**
      a. These are fragments of DNA having different lengths that were created using restriction enzymes. (Can you *see* that in the term?)

C. The DNA RFLP's are loaded into an agarose gel.

D. Turn on the *electricity*. (Remember, DNA is *negatively* charged because of the *phosphate backbone*, so it will be *repelled* on the negative end [Black] and *pulled* by the positive end [Red].) (Electricity should flow from the Black Red strips when performing this process.)

E. The RFLP's will *separate according to length/size* of the fragments.
   1. Big pieces move *slowly* through the gel.
   2. Small pieces move *quickly* through the gel.
   (Good place to use football players. Big heavy linemen do not move as fast as small light running backs.)

F. The DNA fragments are stained for ease of viewing.

G. The DNA bands create a *unique "fingerprint"* of the individuals DNA.

II. Human Genome Project (HGP)

A. The project was begun in 1990 and ended in 2003.

B. The project mapped out the *entire* DNA genome nucleotide sequence for all humans as a *species*.

C. The human genome contains approximately 40,000 different genes. (Please remind students about *rearranging the exons and how that allows humans to create way more than 40,000 proteins/enzymes.)*

D. These 40,000 genes only make up about 3% of the total genome. That is amazing! Only 3%!

E. The other 97% are *control sequences* or introns(*"spacers")*.

III. Transgenic Organisms

A. *Recombined DNA* from two different organisms are combined to make *one* organism that possess traits from *both* "parent" organisms. These traits will be passed on through reproduction as the traits are in the DNA nucleotide sequence, remember. (If you can find a picture of broccoliflower it is a great example. Broccoli is green but it looks like cauliflower in structure.)

IV. Applications ("uses") of DNA Technology:

A. Gene Therapy
   1. This uses a virus to introduce a new gene into a body cell's DNA.
   2. Somatic cells vs. Germ cells (Somatic cells only affect you; germ cells affect future generations.)

B. Pharmaceuticals
   1. Helps with creating new medicines.
   2. Vaccines against diseases and maybe even cancers in the future.

C. Criminal Forensics
   1. DNA fingerprints of suspects.
   2. Paternity/Maternity testing.

D. Environmental Clean-up
   1. Bacteria are used to process human sewage in water treatment plants.
   2. Bacteria that can clean up Oil Spills or breakdown Plastic by eating the oil compounds.
   (These were used in the BP gulf oil spill.)
   3. Organisms helping clean up heavy metals (such as Mercury) from mining or waste collection.

E. Agriculture
   1. Engineering organisms to produce more and larger food.
   2. Genetically engineering organisms able to produce hardier food for easy transport across the world.
   3. Having organisms produce healthier foods.
   4. Having organisms that can produce food during winter. (Winterized)

F. Livestock
   1. Organisms that are "meatier".
   2. Organisms that are "leaner". (Having less fat.)
   3. Organisms that are disease resistant.