Unit 4: DNA Structure & Analysis

- Plasmids are circular, non chromosomal pieces of DNA that can replicate in, and are commonly found in bacteria and simple eukaryotic organisms such as yeast

DNA Structure

- Notes coming soon.

Recombinant DNA Technology

- Notes coming soon.

Gel Electrophoresis

- Agarose gel electrophoresis separates DNA fragments be size
- since DNA fragments are negatively charged, they will drawn towards the positive pole (anode)
- gel is like a molecular sieve where the smaller DNA fragments can move easier than the large ones.
- therefore the rate of movement is inversely proportional to the size of the base pairs.
- Gel electrophoresis sorts proteins and nucleic acids on the basis of their size and/or charge. DNA is negatively charged (phosphates groups) and will migrate toward the positive electrode.
- Longer macromolecules move through the gel more slowly than do shorter macromolecules. The result of this differential rate of movement is a pattern of bands on the gel, each band consisting of macromolecules of one particular size.

Restriction Fragment Length Polymorphism (RFLP)

-first described by geneticist Alec Jeffries in 1985
- RFLP provides unique banding pattern based on the restriction sites present in an individual's DNA
- DNA samples are separated on an agarose gel.
-Restriction fragment length polymorphisms can be used to detect differences in DNA sequences.
-Nucleotide sequences of all but identical twins are different.
-Extracted DNA from a person's cells can be cut up into a set of fragments by exposing the DNA to a series of different restriction enzymes.
-Differences in DNA sequences on homologous chromosomes produce sets of restriction fragments that differ in length and number between different, nonidentical-twin individuals. The differences in restriction fragments produced by this technique are called restriction fragment length polymorphism (RFLPs, pronounced "rif-lips").

-These DNA fragments are of different lengths and will migrate different distances in an electrophoretic gel.

-A genetic marker is any DNA sequence whose inheritance can be tracked. It may or may not be a gene or a sequence within a gene.

-Restriction fragment analysis was used to enable workers studying Huntington's disease to find a genetic marker closely associated with the HD gene.

- Once the genetic marker is known for a particular disease, restriction fragment analysis can be used to test for it.

Restriction Enzymes

- these are natural bacterial defense mechanisms
- acts like molecular scissors, making cuts at specific sequence of base pairs that it recognizes
- these enzymes destroy DNA from invading viruses, or bacteriophages (phages)
- phages are viruses that infect and destroy bacteria.
- bacterial restriction enzymes recognize very specific DNA sequences within the phage, cut it out making it the phage harmless.
- they are named for the bacteria for which they were isolated.
- named 'restriction enzymes (before scientists knew how they functioned) b/c they would restrict the growth of phages
- the enzyme cuts or chemically separates the DNA molecule at a particular site called the **restriction site.**
- the enzyme can cut at more than one site
- if site is linear and cuts in two spots – the result is three fragments
- if circular and cut at two pots, the result is two fragments (similar to cutting an elastic band.
- when restriction enzymes are used to cut strands of circular plasmid DNA (such as what is used in this kit), the fragments can be observed using gel electrophoresis.

DNA Fingerprinting

- scientists use a radioactive complementary DNA probe that will recognize and bind to that sequence.
- allow biologists to locate, identify, and compare the DNA of different individuals.
- probe can be describe as a 'radioactive tag' that will bind to a single strand DNA fragment and produce a band in a gel or band on a piece of blotting membrane (also known as a **Southern Blot**)
- the evidence needed for DNA fingerprinting can be obtained from any biological material that contains DNA – body tissue, body fluid, hair follicles etc.

- If sample too small, it can be amplifies using PCR techniques