Chapter 13
DNA Technology

- Genetic Engineering – the application of molecular genetics for practical purposes. Can be used to IDENTIFY or TRANSFER genes.
- DNA Technology – can be used to cure diseases, treat genetic disorders, improve food crops, etc.
- Restriction Enzymes – bacterial enzymes used to "cut" DNA molecules into more manageable pieces.
  - they recognize a specific nucleotide sequence
  - "cut" the DNA at a specific site within the sequence.
  - "sticky ends" (single chain segments or tails created on the cut piece of DNA….easily bind to complementary strands of DNA.

  ** Pieces of DNA cut with the same restriction enzyme can bind to form a new sequence of nucleotides…..therefore, DNA HAS BEEN TRANSFERRED OR ISOLATED!!!!!!!

See fig.13-1 on pg. 239.

- If restriction enzymes are used to ISOLATE a gene, it can be transferred to an organism via a CLONING VECTOR.

- Cloning Vector – a carrier that is used to clone a gene and transfer it to another organism.
- Plasmid – a ring of DNA found in many bacteria in addition to its main chromosome. Can be used in gene transfer in the following manner:

  1. Plasmid is removed from a bacterium.
  2. Using restriction enzymes, the plasmid is cut.
  3. A donor gene(specific isolated gene from another organism) is spliced into the plasmid.
  4. Plasmid is returned to the bacterium where it replicates as the bacterium divides….thus cloning the donor gene = GENE CLONE
  5. Bacteria can now be used to "infect" other organisms – transferring the gene to them. Exs. See fig. 13-3 on pg. 240 and 13-4 on pg. 241.

- Genomic Library – The set of 1000's of DNA pieces from a genome produced from the use of restriction enzymes. Several libraries can be made from the same genome, depending on types of restriction enzymes used. Some of the DNA pieces will contain specific genes that can be transferred, if desired.
- Recombinant DNA – the combination of DNA from 2 or more sources.

- Transgenic Organism = a host cell that receives the recombinant DNA. This is then cultured so that it reproduces many times….thus the DNA is cloned. Ex. Bacteria carrying the gene for human insulin can now be used to produce large quantities of insulin.
- Expression of Cloned Genes Can Be Difficult – not all of the cell's genes are expressed – especially foreign genes. There are 2 ways to induce expression…

  1. Transfer, along with the foreign gene, the promoter sequences that turn the gene on.
  2. Insert the foreign gene beside a gene that is normally expressed in large quantities within the host cell. Hopefully the foreign gene will be expressed along with the frequently expressed gene.

- DNA Fingerprint – a pattern of bands made up of specific fragments from an individual's DNA.
- DNA fingerprints can be used to:

  1. Compare 2 individuals to see if they are related.
  2. See if 2 different species are closely related.
  3. To identify blood/tissue samples at a crime scene. See fig. 13-5 on pg. 243.

Steps in Making a DNA Fingerprint:

- 1. RFLP Analysis – (Restriction Fragment Length Poylmorphism) – extract DNA from a specimen and cut it into fragments using restriction enzymes…..The number and length of the fragments will vary from person to person.
- 2. Gel Electrophoresis – technique that separates the DNA fragments according to size and electrical charge.

  a. Samples to be compared are placed into wells made in the gel.

  b. Electrical current is run through the gel.

  c. Negatively charged DNA fragments move toward the positive electrode and vice-versa.

  d. Pores in the gel cause smaller DNA pieces to move faster/farther across the gel. This causes pieces to separate out at different locations on the gel (bands are formed at different locations). See fig. 13-6 on pg. 244.

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e. DNA pieces on gel are “unzipped” and blotted onto filter paper.

f. PROBES are added to the filter paper = radioactive segments of DNA complementary to those being fingerprinted.

g. Probes bind to the DNA in the samples and form visible bands when exposed to photographic film. This is known as a DNA FINGERPRINT!!!

- Not All DNA is Fingerprinted – just the repeating sequences found in the non-coding part of every person's DNA. Only 5 sites are compared. This is very accurate. Less than 1 chance in a million that all 5 sites will match between 2 people.
- Polymerase Chain Reaction – (PCR) – a technique used to multiply the amount of DNA from a very tiny sample, so that a DNA fingerprint can be made even when the original sample does not supply does not supply enough. This helps a lot when working a crime scene!!
- Materials List for PCR – a DNA sample, a supply of the 4 DNA nucleotides, DNA polymerase, and primers (artificially made single stranded piece of DNA needed to start replication).
- When PCR materials are incubated, the sample of DNA doubles providing ample supplies for DNA fingerprinting. See fig. 13-7 on pg. 245.

- NOTE – RFLP analysis requires 5,000 to 50,000 cells from a sample. Good when sample size is large. PCR can work with a sample as small as 50 cells. This is great when the sample is very small.
- Human Genome Project – World wide scientific effort. Has successfully mapped approximately 3 Billion nucleotide pairs, OR about 100,000 genes. It was started in 1990 and finished 5 years ahead of schedule. It may help improve diagnosis, treatments, cures for genetic disorders.
- Gene Therapy – treating a genetic disorder by introducing a gene into a cell, OR correcting a gene defect in a cell's genome. Read top of pg. 248.
- Ethical Issues – resulting from the genome project:
  1. Will insurance companies refuse coverage based on genetic deficiencies a person may have?
  2. Will employers discriminate based on genetics?

- Medicines produced by DNA technology – See table 13-1 on pg. 249.
- Genetically Engineered Vaccines:

  1. Traditional vaccines contain "treated" pathogens (disease causing viruses/bacteria). These vaccines cause immunity, but there is a small chance of getting the disease if the vaccine is not prepared properly.

  2. Genetically engineered vaccines, such as the one for Hepatitis B, make use of viral genes transferred into harmless agents such as yeast cells. This causes immunity, but there is no chance of getting the disease from the vaccine because the original whole virus is not used. See fig. 13-9 on pg. 250.

- Increasing Agricultural Yields:

  1. Plants that produce toxins harmful to insect pests. Ex. Tomatoes resistant to hornworms.

  2. Plant strains resistant to herbicides make it easier/cheaper to control weeds. Exs. Wheat, cotton, soybeans.

  3. Disease resistance in certain plants.

- Crops that do not need fertilizer:
  - plants require nitrogen to grow.
  - LEGUMES (beans, peas, alfalfa, clover) – have bacteria in their roots that take nitrogen out of the air and put it into the soil = NITROGEN FIXATION.
  - other NON-legume type plants require expensive nitrogen fertilizer
  - scientists are working to transfer genes from legumes to other crops.

- Concerns about genetic engineering:

  1. Will engineered crops contain toxins or cause allergies?
  2. In general, engineered crops can be sold without special permits/labels.
  3. FDA requires that foods containing genes from known allergens (such as peanuts) be proven safe. Proof also required for foods that contain a new protein/fat/carb.
  4. Will engineered crops spread into the wild and wipe out native plants?
  5. Will new “superweeds” be created?