UNIT 6: DNA/RNA/PROTEIN SYNTHESIS

TOPIC 4 – GENES AND TECHNOLOGY

By the end of this topic, you should be able to…

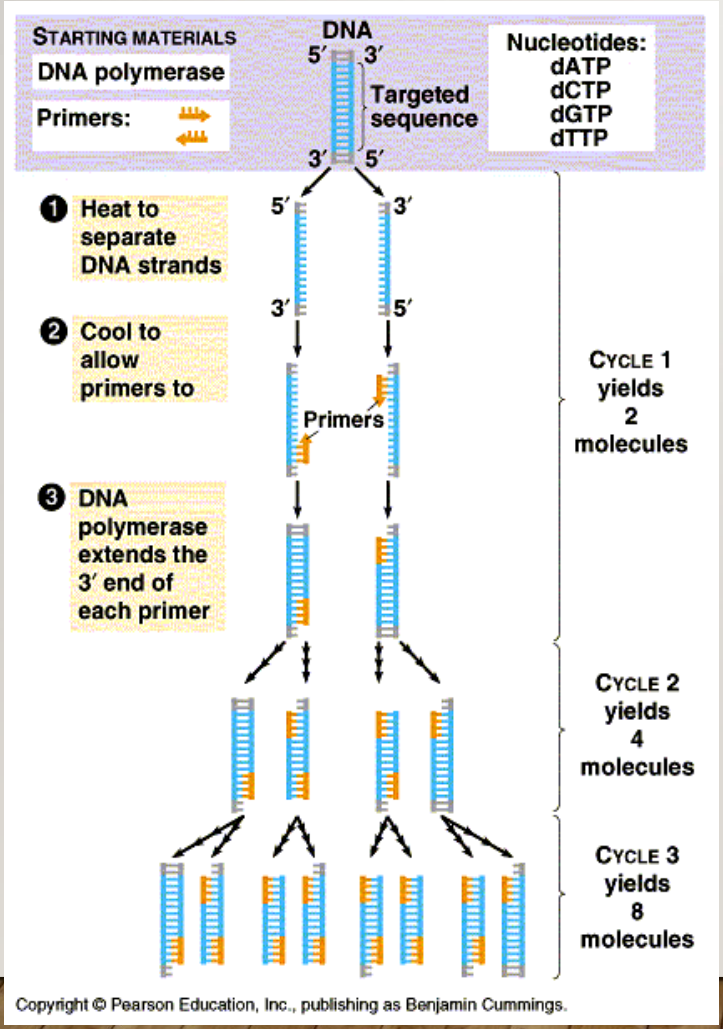
- *Describe the purpose and methods of gel electrophoresis and analyze electrophoresis results*
- *Provide examples of the practical uses of biotechnology, including insulin production and cloning*
- *Describe the purpose and methods of PCR (polymerase chain reactions)*

GENETIC ENGINEERING =MANIPULATING DNA

- **With present technology and knowledge of DNA structure, we can extract, identify, modify, copy,** and transfer DNA sequences!

Genetic engineering allows scientists to create desirable traits within organisms to meet specific needs without relying on natural mutations.

PCR



- PCR is the artificial replication of DNA in a controlled environment.
- We use heat to separate the strands and special heat-resistant bacterial enzymes to speed up the process

EXTRACTING DNA (FIRST STEP TO GENETICALLY ENGINEER AN ORGANISM)

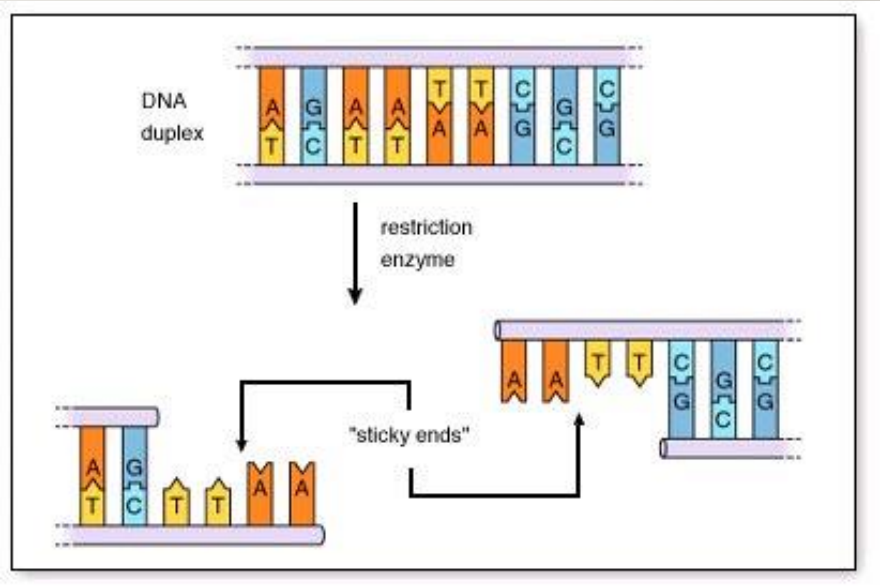
DNA extraction is relatively easy to do from the cells of plants and animals! We can even do it in the classroom!

Once you have extracted the DNA, you can copy, change, and transfer it in helpful ways.

CUTTING DNA (SECOND STEP)

Remember: DNA is a very long molecule

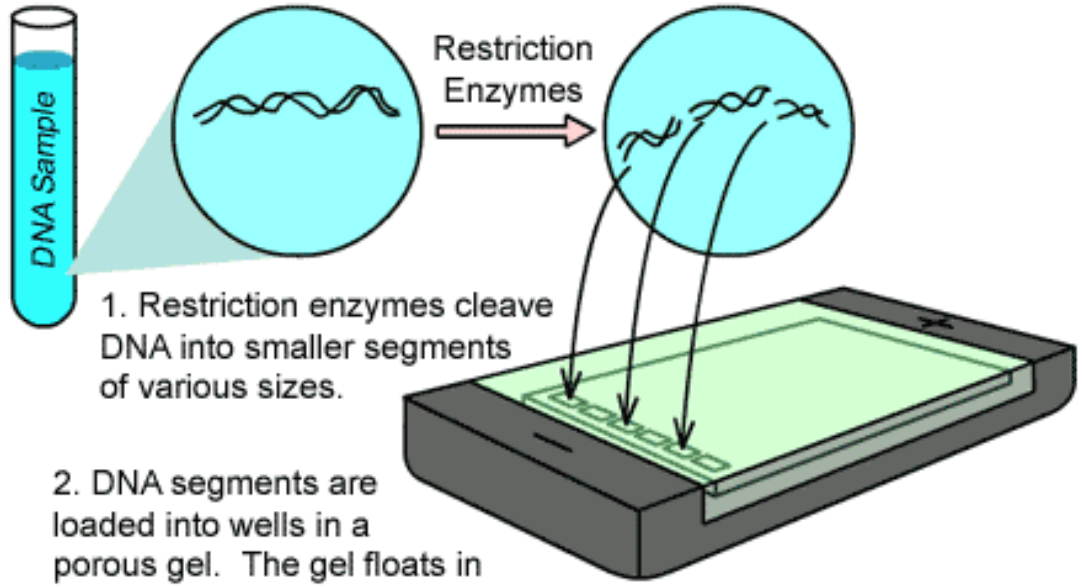
- In order to make working with DNA more manageable, scientists use restriction enzymes to cut DNA into fragments known as restrictive fragments.
- These **<u>restrictive enzymes</u>** are chemicals that bind to and make cuts at specific sequences in DNA.



SEPARATING DNA (3RD STEP)

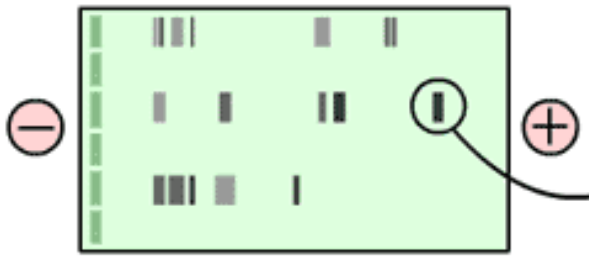
- Once DNA is cut into fragments, scientists select only those sequences that code for particular traits.
- **Gel electrophoresis** is one technique that is used to sort DNA sequences by **size**, which can be read and analyzed.
- Once it is sorted, scientists can…
  - study **individual genes on the DNA**
  - obtain a segment of DNA to **copy** using a technique called **PCR** (polymerase chain reaction)
  - help locate **genetic diseases** to potentially **eliminate** them

Figure S-2: Gel Electrophoresis



1. Restriction enzymes cleave DNA into smaller segments of various sizes.

2. DNA segments are loaded into wells in a porous gel. The gel floats in a buffer solution within a chamber between two electrodes.



3. When an electric current is passed through the chamber, DNA fragments move toward the positively-charged cathode.

4. Smaller DNA segments move faster and farther than larger DNA segments.

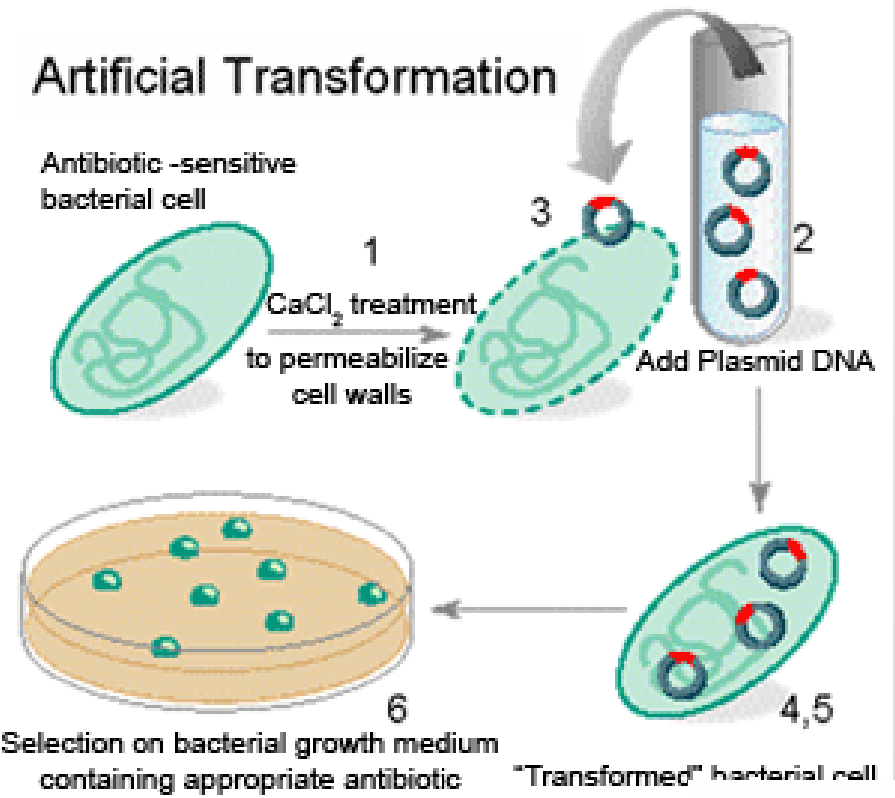
CHANGING DNA (4TH STEP)

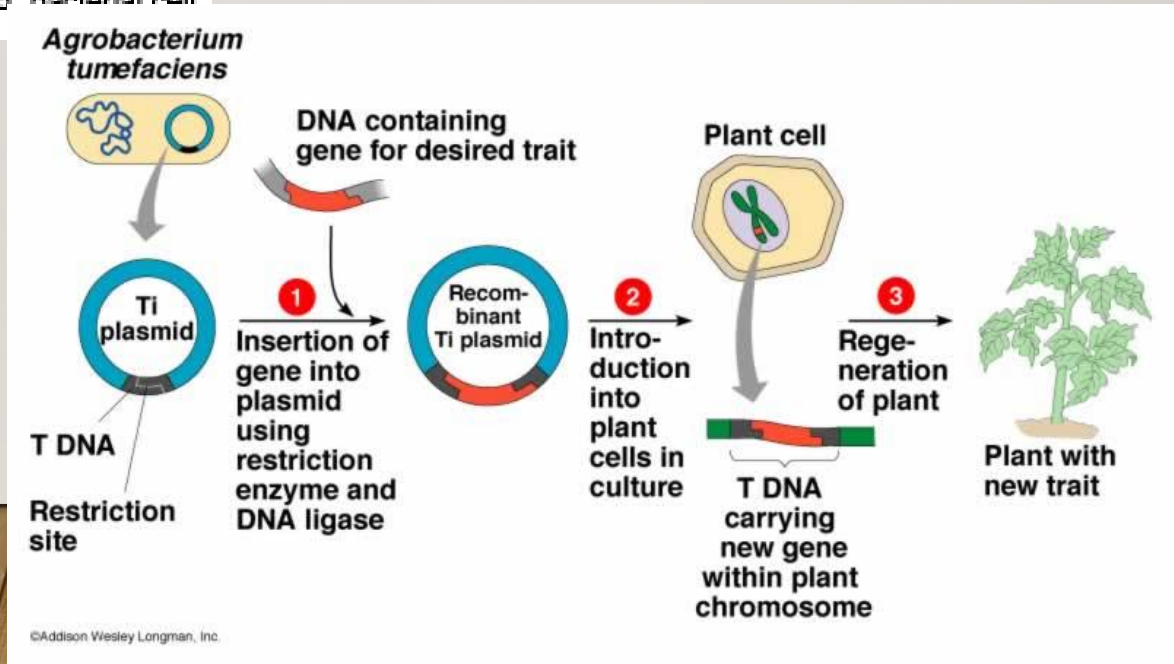
Like Frederick Griffith's early bacterial transformation, scientists are able to take segments of one organism's DNA and place it into the genome of other organisms

- **Recombinant-DNA Technology** is the type of genetic engineering where DNA from two are more different sources is **joined** (resulting in what are called **transgenic organisms**)

TRANSFORMING BACTERIA-THE STEPS

- **Recombine** DNA-restriction enzymes like *Eco*RI cut the DNA into fragments to prepare them for recombination
- **Transport DNA-a** scientist has to **insert** recombinant DNA pieces into the host cell (into plasmids, or circular DNA, in bacterial cells)
- **Transfer DNA-** When the host cell divides (by binary fusion), it also makes a copy of the newly transformed plasmid (called a recombinant plasmid)
- **Genetic markers** , such as those for antibiotic resistance, inserted into the plasmid along with the specific desired gene allow scientists to pinpoint transgenic cells


Artificial Transformation
Antibiotic -sensitive bacterial cell
1
$CaCl_2$ treatment to permeabilize cell walls
3
2
Add Plasmid DNA
4,5
"Transformed" bacterial cell
6
Selection on bacterial growth medium containing appropriate antibiotic


Agrobacterium tumefaciens
DNA containing gene for desired trait
Plant cell
Ti plasmid
T DNA
Restriction site
1
Insertion of gene into plasmid using restriction enzyme and DNA ligase
Recom-binant Ti plasmid
2
Intro-duction into plant cells in culture
3
Rege-neration of plant
Plant with new trait
T DNA carrying new gene within plant chromosome
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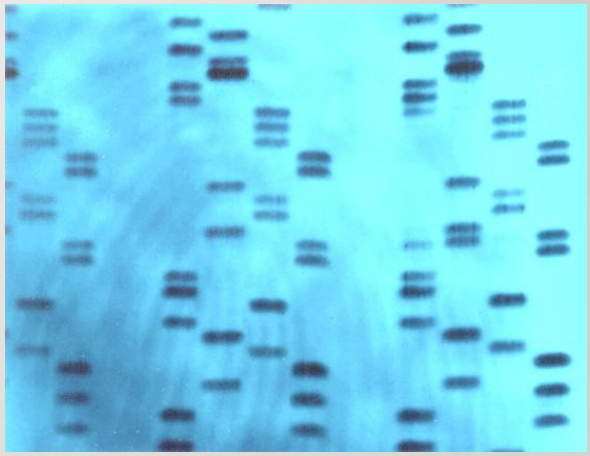
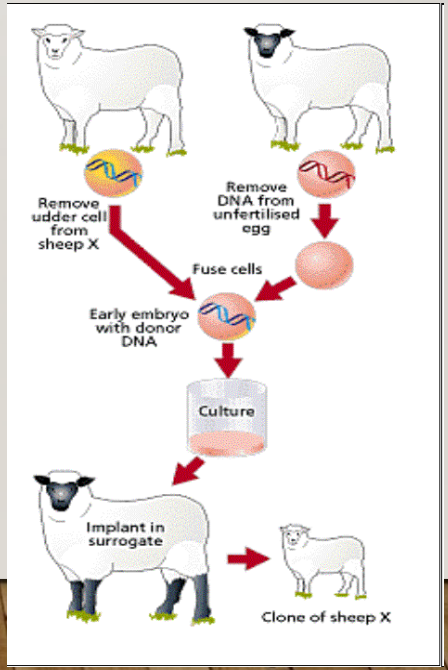
USES OF RECOMBINANT-DNA TECHNOLOGY

- **Advances in medicine**
  - Transgenic animals and plants that provide health benefits
  - Transgenic animals used as test subjects
  - Insulin or Human Growth Hormone production by bacteria
  - Human Genome Project & Gene Therapy
- **Agriculture**
  - Genetically modified plants and animals (their cells don't accept foreign DNA very well so you must **infect** plant and animal cells with bacteria containing recombinant plasmids)

- **Personal identification**
  - DNA fingerprinting
  - Paternity testing
  - Forensic science
- **Cloning**
  - When humans clone, they use a single cell of an adult organism to grow a new genetically identical individual
  - They Inserts the nuclei from the blastula stage (hallow ball of cells after several divisions of a zygote) of an embryo into an adult cell

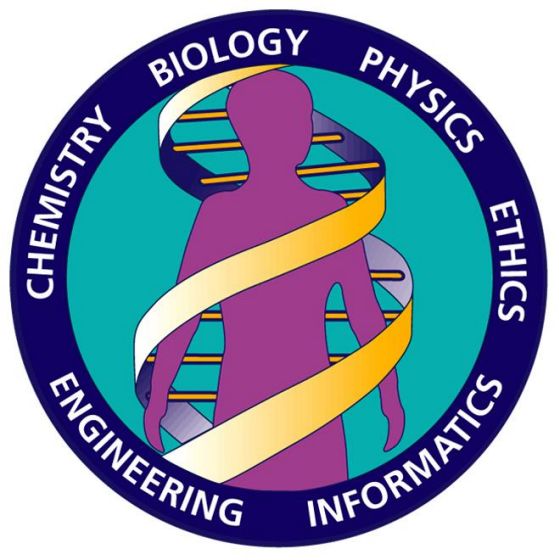
    Ex. Dolly the sheep





HUMAN GENOME PROJECT

- *The goal was to determine the sequence of nitrogen bases in human DNA. An entire set of DNA from a body cell is considered that organism's genome.*



- *There are 3 billion base pairs in the human genome and approximately 25,000 genes.*
- *NIH is striving to cut the cost of sequencing an individual's genome to $1,000 or less. Having one's complete genome sequence will make it easier to diagnose, manage and treat many diseases.*