Experimenting with DNA is nothing new. Nature has been performing DNA experiments for close to 4 billion years, in the form of mutations, recombinations, crossing over of chromosomes etc.

- *This is genetic basis of evolution and the diversity of life.*
- *In the latter part of the 20th Century, humans have been **purposefully** bringing about genetic changes through **RECOMBINANT DNA TECHNOLOGY** (a major part of **genetic engineering**).*
- *The "recombining" of the DNA from different species has allowed the production of rare proteins in large quantities (e.g. insulin). Isolating, modifying, and reinserting DNA sequences, called "Human gene therapy" is aimed at controlling or curing genetic disorders.*
- *As DNA is so fundamental to life processes, DNA technology raises many social, legal, ecological, and ethical questions.*

Our understanding of DNA and new technology allow us to isolate, cut, and splice together gene regions from different species.

- **Recombinant DNA** (**rDNA**) is DNA in which **DNA from one species** (e.g. a gene coding for the protein Insulin) is inserted **into the DNA of a second species** (e.g. bacteria). The second species can then go on to produce proteins of the first species, and when it reproduces, it will copy the other species' DNA and pass it onto its offspring. The gene can now said to be "**cloned**." We can also make many copies of the DNA ("amplify") that interest us.
- The genes, and in some cases their protein products, are made in quantities that are large enough for research and for practical applications***. The uses of recombinant DNA include***:

1. **CLONING GENES**: viruses and bacteria can be used to make copies of the gene(s) of another species. Huge amounts of any piece of DNA can now be made using a technique called the **Polymerase Chain Reaction** (**PCR**)
   - **Whole organisms** can now be cloned as well!
2. **PRODUCING BIOTECHNOLOGY PRODUCTS**: genetically engineered prokaryotic and eukaryotic cells can be used to **mass produce** once rare medicinal **proteins** and **hormones** as well as **vaccines** to prevent disease (e.g. hepatitis B).

- The future may provide vaccines for such things as herpes and AIDS and many other diseases.
- it allows us to **produce large amounts of proteins** that are very difficult to get otherwise or are usually present only in small quantities from natural sources.
  - e.g. **human growth hormone -** once took 50 pituitary glands from cadavers for a single dose. Can now be made in mass quantities, and much less expensively.
  - **Insulin** used to come from the pancreatic glands of cows and pigs. This was expensive and much less pure than the cloned human DNA available today.
  - **tPA (tissue plasminogen activator)** - a protein that activates an enzyme that dissolves blood clots, normally present in only tiny amounts, can now be made in large quantities and is routinely used to **dissolve the coronary blood clots** of heart attack victims.
- Other Biotechnology Products include these medicines and Vaccines:

Factor	Used to Treat
Interferons	Cancer
Erythropoietin	Anemia
Interleukin-2	Cancer
Blood Clotting Factor VIII	Hemophilia
Human Lung Surfactant	Respiratory Distress Syndrome
Atrial natriuretic factor	High Blood Pressure
Tumor necrosis factor	Cancer
Vaccines	• AIDS • Herpes • Hepatitis A, B, C • Lyme Disease • Whooping Cough • Chlamydia • Many animal vaccines

3. **MAKING TRANSGENIC ORGANISMS**: we can alter the DNA of bacteria, plants, and farm animals to make them more valuable and less susceptible to disease.

- ***Bacteria** is very useful in this capacity*

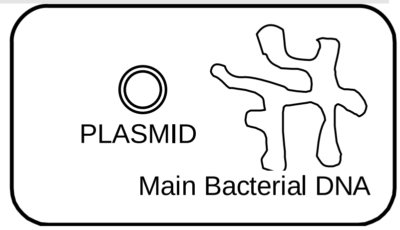
    - Bacteria are used to ***Protect and Enhance Plants***: e.g. protect plants from frost, provide more nitrogen to roots, even produce insecticides to kill insects.
    - Bacteria are used for ***Garbage Disposal***: Bacteria can be engineered to eat toxic waste and clean up oils spills, filter the air, remove sulfur from coal etc. (***bioremediation*** is the name for using organisms to clean up man's messes)
    - Bacteria are used to ***Produce Chemicals***: e.g. genetically engineered bacteria produce phenylalanine, used in the production of 'Nutrasweet' artificial sweetener.
    - Bacteria are used to ***Process Minerals***: genetically engineered bacteria can be used to extract greater amounts of metals (e.g. U, Cu, Ag) from low-quality ores.
- ***<u>Transgenic plants</u>***:
    - Are already widely used in **agriculture**. The bacterium *Agrobacterium*, which naturally infects many plants, is used, as well as artificial vectors called *protoplasts*.
    - Over **50 types of genetically engineered plants** today. Contain new genes that help **resist insects**, **viruses**, or **herbicides** (e.g. "*Roundup*" resistant wheat). Genetically engineered soybeans, cotton, alfalfa, and rice are already on the market.
    - Transgenic plants can also be made that are **resistant to temperature extremes**, **drought**, and **salty** soils.
    - There are transgenic plants that resist **spoiling** and **bruising**.
- ***<u>Transgenic animals</u>***:
    - by injecting DNA that codes for the uptake of **bovine growth hormone** (bGH) into the eggs of fish, cows (e.g. 25% greater milk production), pigs, rabbits, and sheep, bigger animals can be produced more cheaply.
    - "***Gene Farming***" refers to the use of transgenic farm animals to produce pharmaceutical drugs like human lactoferrin (absence of the gene for lactoferrin in humans causes reoccurring bacterial infections of the intestine). Transgenic cows, for example, will produce human lactoferrin in their milk. Drink the milk, and you get protection from the intestinal infections.
    - There are plans to produce drugs to treat cystic fibrosis, blood diseases, and cancer through this method.

4. <u>GENE THERAPY</u>

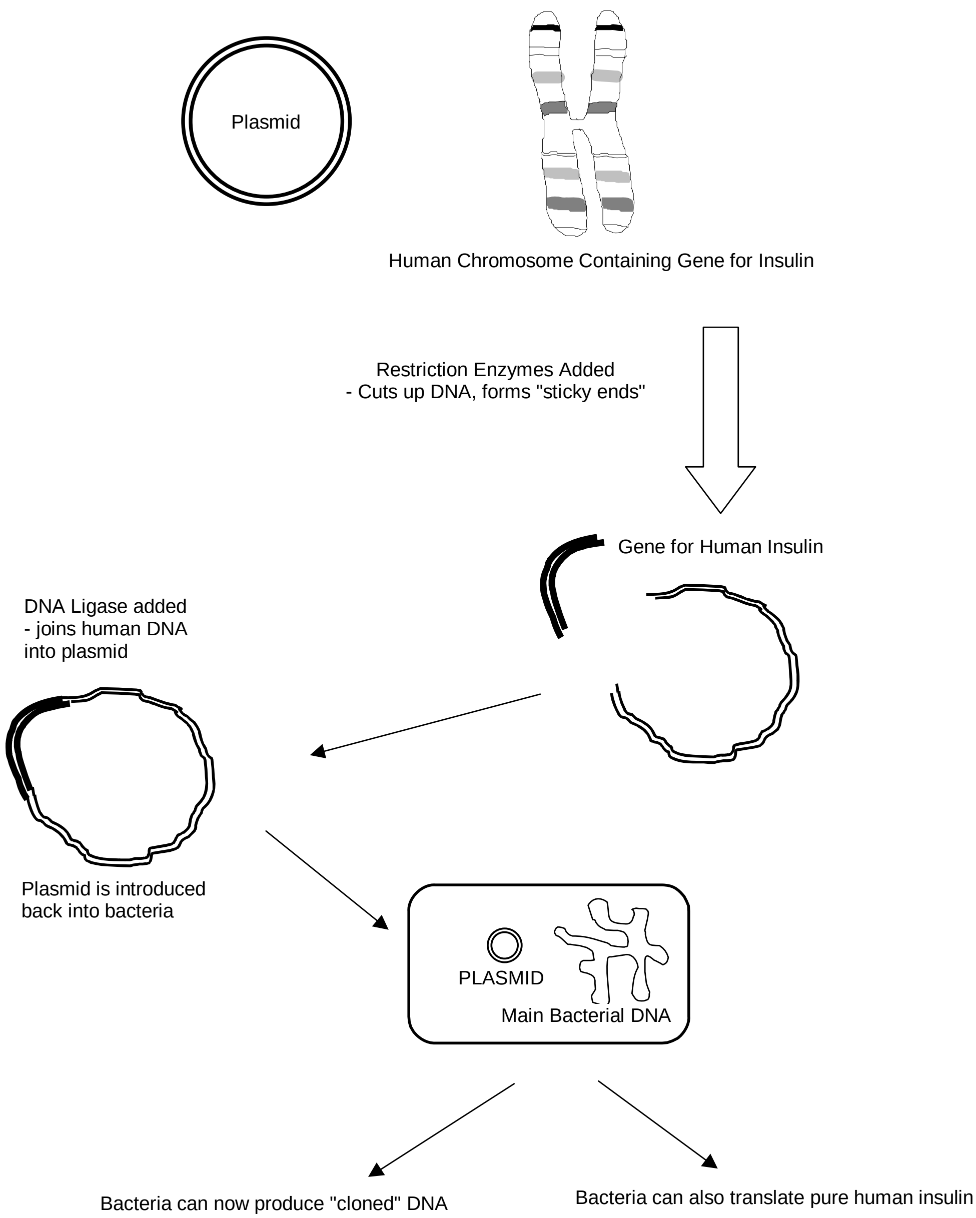
- The idea behind gene therapy is to replace defective genes in a living organism (especially humans) with healthy genes, and is used to treat genetic disorders and diseases.
- Can be "**Ex Vivo**" (outside living organism) or "In **Vivo**" (inside living organism).
- In **Ex Vivo** gene therapy, cells are removed from the patient, treated, then returned to the patient.
    - Can use retroviruses to introduce the nucleic acid into the cells to be treated.
    - e.g. has been used to treat *SCID* (severe combined immunodeficiency syndrome **-** sufferers lack an enzyme needed for certain white blood cells). Insert the correct gene into patient's WBC or stem cell and reinsert.
    - e.g. **-** has been used to treat liver cells in *hypercholesterolemia* (in this disorder, liver is unable to remove cholesterol from blood ⇨ heart attacks)
    - e.g. **-** used to make *cancer patients* more resistant to chemotherapy drugs)
- ***In Vivo*** techniques introduce genes right into the bodies of patients.
    - e.g. an inhalant spray containing an adenovirus containing a gene to treat cystic fibrosis (must be reapplied regularly).
    - e.g. treat or cure Parkinson's Disease by grafting dopamine-producing cells right onto the brain.
    - e.g. treat hemophilia with regular injections of cells that have the normal clotting-factor genes.
    - e.g. work is being done to see if retroviruses can be used to carry genes for *cytokines* (soluble hormones of the immune system) to treat cancer.
    - "*antisense*" technology may be used to turn off cancer-causing genes (oncogene) or turn off AIDS viruses.

Techniques of Genetic Engineering

Making Recombinant DNA



- A "**<u>vector</u>**" is something that can get the DNA from one species into the other species' DNA. Often, this can be a "**<u>plasmid</u>**", a circular piece of DNA found in some bacteria.
- A **human gene**, such as the gene for **<u>insulin</u>**, is inserted into the plasmid and then the plasmid is **taken up by bacteria**. The bacteria reproduces the plasmid along with its own DNA when it reproduces, and translates the **human gene**, producing **human protein**.

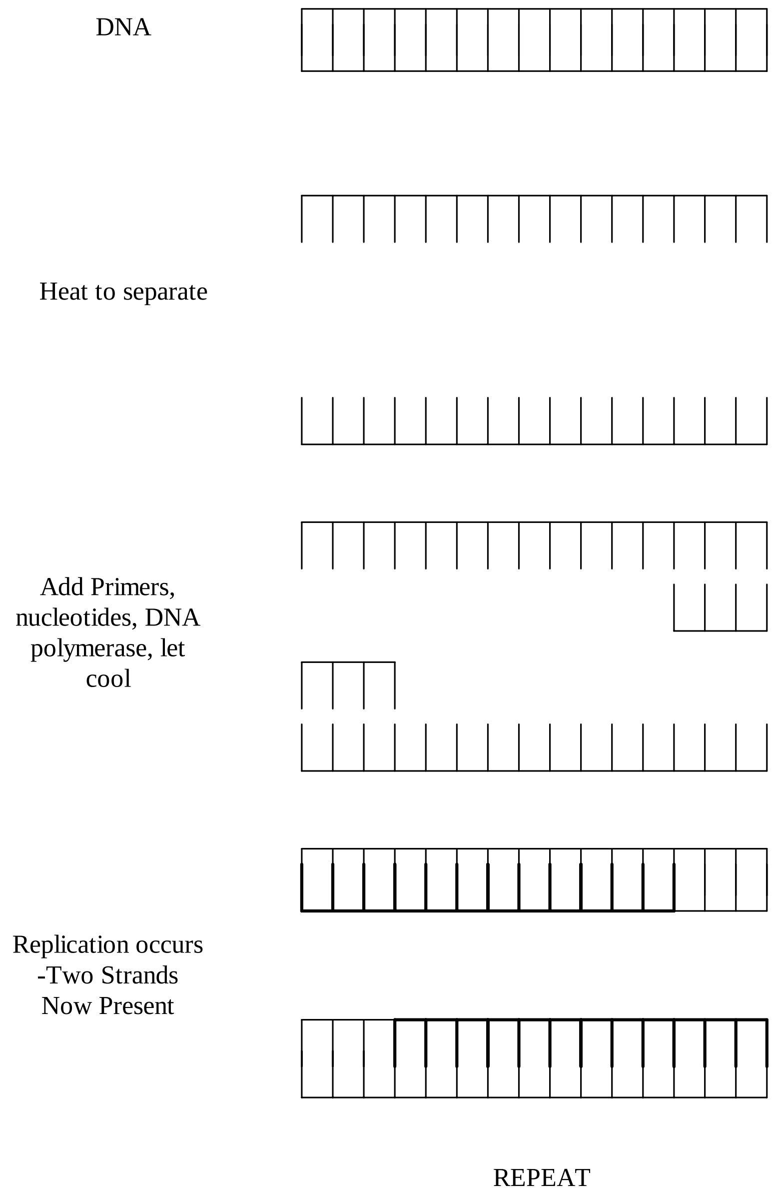


- This technique can be used to produce "cloned" DNA by allowing the bacteria to multiply themselves.

Polymerase Chain Reaction

- Another technique, called the **<u>Polymerase</u> <u>Chain</u> <u>Reaction</u>**,   (**PCR**) can also make large amounts of a desired gene or piece of DNA. PCR can be done without bacteria, inside test tubes, and can amplify billions of times samples with very little DNA (e.g. a single hair from a crime scene, or inside some fossils).
- PCR makes huge amounts of any gene, quickly.

1. heat the DNA to about 93ºC, which unwinds the DNA and separates the two strands.
2. Add some replication primers, and allow to cool
3. Add heat resistant DNA polymerase (the replication enzyme) and free nucleotides. The DNA will copy itself.
4. Heat and repeat. The DNA will go on doubling itself each "generation".



- After PCR has been performed, the **sequence of bases** on DNA can be determined (e.g. using the "Sanger Method"). Sequencing bases is useful for:
  - study evolutionary relationships between organisms (e.g. humans and chimpanzees), and trace the origin of human races.
  - map every single nucleotide on all human chromosomes ("the **Human Genome Project**")

- PCR amplified DNA can be **analyzed** using a **<u>DNA probe</u>** (a specially synthesized single strand of radioactive DNA nucleotides) that will bind to a complementary DNA strand on the DNA being tested. This can be used to ***detect viral infections***, ***diagnose genetic disorders***, and ***diagnose some cancers***.

- A quick way of comparing the DNA from two different organisms is by using **<u>RFLP ANALYSIS</u>**. (Restriction Fragment Length Polymorphisms). This can provide a "**DNA fingerprint**" that is unique to each individual (*except* identical twins). RFLP analysis uses specific restriction enzymes that cut DNA at specific sequences. This produces fragments that, when separated using **gel electrophoresis**, produce patterns of bands that can be compared to another person's pattern of bands.
- If the band pattern is identical, the DNA must have come from the same person.
- This can be used to **identify a criminal** from a blood or semen stain. It can also determine who the **father** of a child is, with a high degree of accuracy.
- RFLP analysis is also used to see whether a person **carries a gene for a genetic disorder** like cystic fibrosis or sickle-cell anemia, and can be used for prenatal diagnosis.
- RFLP analysis also contributes to our knowledge of evolution and evolutionary relationships by comparing human and animal DNA.