

Genetic Engineering

1977 – Frederick Sanger discovered the complete base sequence for one type of virus, identified all 9 of its genes, and became the first to do so. This opened up a whole new world for genetic procedures and study.

Genetic Engineering – the changing of an organism’s genome to serve some purpose – is still based on **three** important early discoveries:

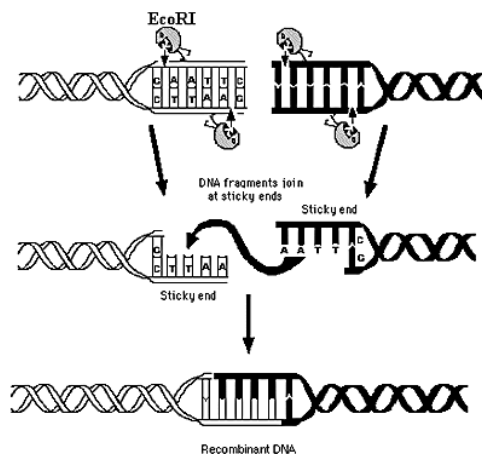
1. A way to break DNA at certain specific chosen spots, not just randomly (the use of **restriction endonucleases**)
2. A process to copy DNA over and over again in the lab (called **DNA amplification**)
3. A way of sorting different size DNA molecules (called **gel electrophoresis**)

RESTRICTION ENDONUCLEASES

- Are enzymes made by prokaryotic cells (like bacteria)
- There are many types
- Each type recognizes a certain base sequence of DNA and cuts it at that point – called a **restriction site**

Two reasons why these enzymes are useful:

1. We know exactly where they are going to cut the DNA, and they cut the same way every time, making smaller evenly sized DNA pieces called **restriction fragments**
2. They cut the DNA in a way that leaved a few unpaired bases sticking off (called a sticky end), that we can use to join pieces of DNA back together in new ways. This is called **recombinant DNA** .



**Restriction Enzyme
Action of EcoRI**

DNA AMPLIFICATION

This is the process of making a large sample of DNA from a small sample. Accurate copies are made over and over. There are **two** main ways this can be accomplished:

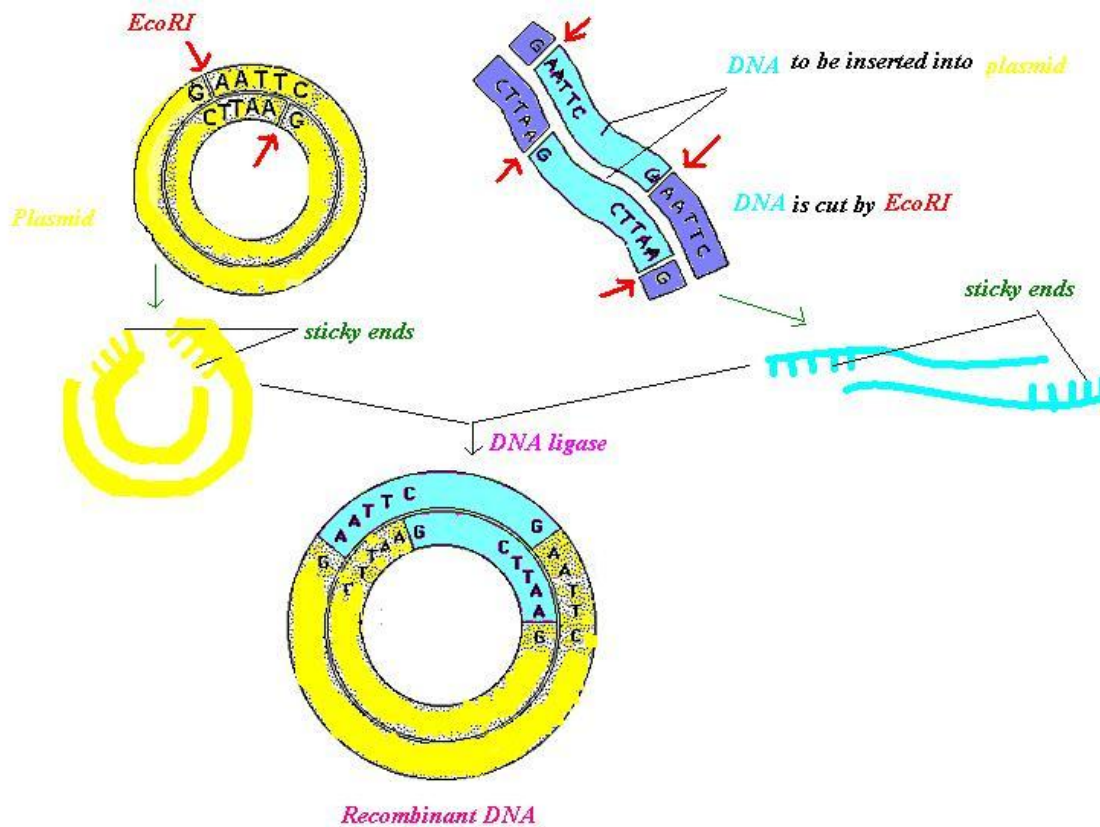
- 1) Using a **Vector** - getting bacteria to multiply the sample for you.

Treat the bacteria with the same restriction endonuclease used to make the fragment of DNA you need to copy. It will cut the bacteria in the complementary place.

Since the sticky ends are complementary, the DNA sample fragment will become part of some of the bacteria. (doesn't always work with all bacterial cells)

Separate the bacteria that do incorporate the DNA fragment from those that didn't.

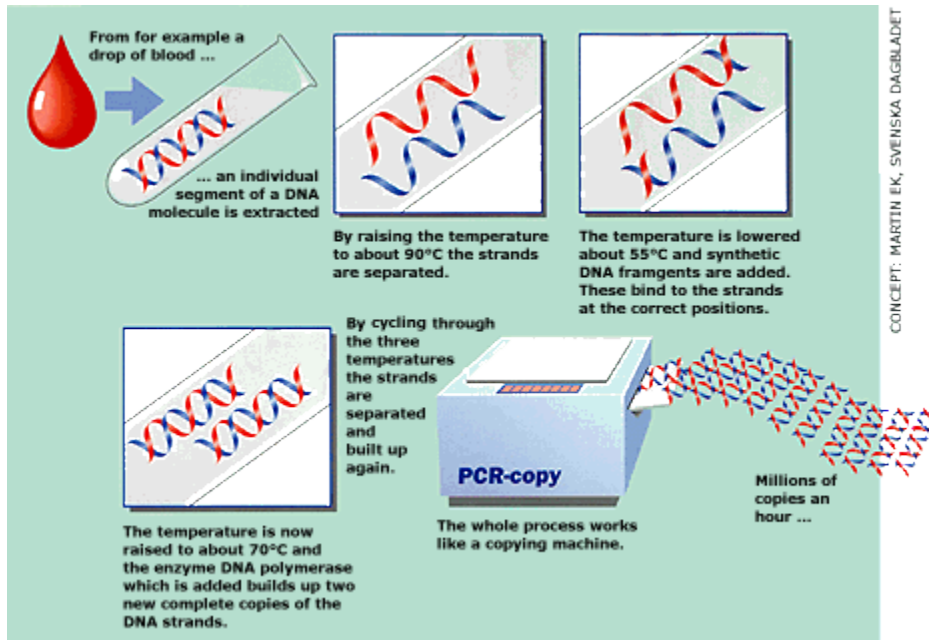
Let those new recombinant cells reproduce through mitosis.



2) Using Polymerase Chain Reaction Method (PCR)

(This method won a Nobel Prize in 1986!)

Your tiny DNA fragment sample is put in solution with lots of nucleotides and DNA primers. Your sample unzips. The DNA primers attach and use the nucleotides floating around in the solution to build complementary strands. You now have two copies of your fragment. Repeat the process to get 4 copies, then 8, then 16, ... If your sample contains several thousand fragments, you can make billions fast!



GEL ELECTROPHORESIS

A sample of different sized fragments can be separated into groups based on the fragment mass and electrical charge.

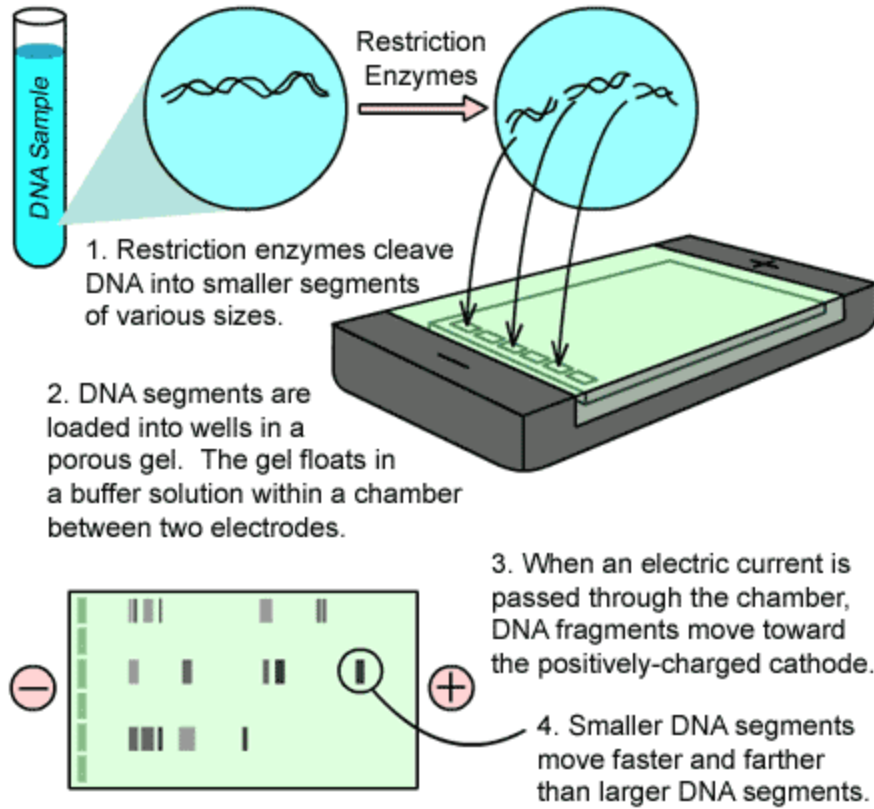
Here's how it goes:

Make a batch of conductive gel, pour it into plastic trays, let it set, and punch a series of depressions (called wells) in one end.

Put the gel plate into a chamber, put liquid in, add your cut up sample into the wells.

Add electricity, the fragments will separate to make a distinctive DNA fingerprint pattern of bands in the gel. Smaller fragments move faster and farther than the larger, heavier DNA segments.

Figure S-2: Gel Electrophoresis



How do we use all this stuff?

- ❖ Crime Scene Investigations - single hair follicles, blood, semen, epithelial cells(skin), sweat, anything that may provide even the smallest sample can be cut up, copied and analysed, and the perpetrator or victim identified
- ❖ Identifying the unidentifiable- DNA samples from possible relatives or a sample from earlier in life might be used to identify a body when there is extreme trauma
- ❖ Identifying parents- “You ARE the father!”
- ❖ Genetically modifying plants and animals (increased crop growth, artificial hormones, PCB/oil-eating bacteria, plants that glow near land mines!

GENETICALLY MODIFIED ORGANISMS (GMOs) and GENETICALLY MODIFIED FOODS (GMFs)

There are a number of situations where humans have changed whole organisms, usually bacteria, or food crops in order to meet our needs. Some examples include:

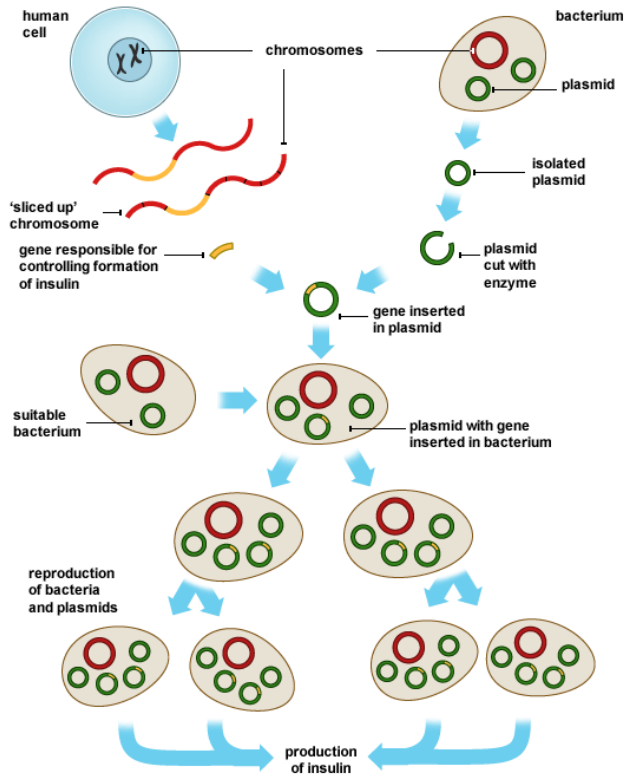
- ❖ In some US cows, extra hormone injections have been done, by copying growth hormone (somatotrophin) using bacterial vectors. When this is injected into the cows it makes them grow bigger and produce more milk. It is NOT approved for use in Canada, despite companies trying for years.



- ❖ Over 50 different GM plant species have been approved and are growing in Canada already, including over 50% of the corn and canola crops. An example of one of the ways crops can be modified would be like herbicide resistant corn. Herbicides kill weeds, which interfere with crop production, but many herbicides will kill all plants, not just the weeds. By taking a herbicide resistant -gene from bacteria, putting it into corn gametes, it makes herbicide resistant corn. Then when the crops are sprayed with the herbicide, the weeds will die, but not the corn.



- ❖ Making artificial human hormones – at one time, hormones needed for use as medicine, like insulin for diabetics, had to be extracted from farm animals. This caused a problem because some people were allergic to the animal hormones. These days human insulin is produced by modified bacteria, lowering the cost and eliminating allergic reactions.

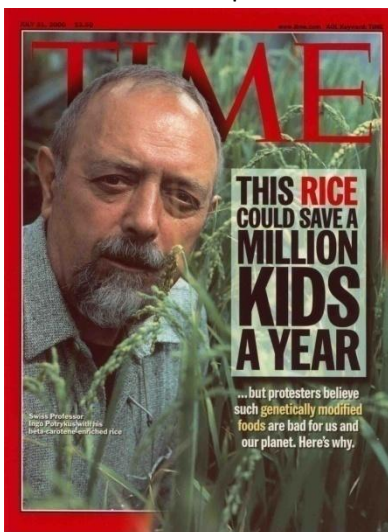


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❖ Bioremediation – using modified organisms for environmental clean-up

- PCB-Eating or Oil- Eating bacteria can be used for cleaning oil spills or PCB spills

❖ “Golden Rice” is fortified with extra beta carotene and iron, to make it more nutritious. It was then distributed to poorer countries to provide better nutrition with less effort and land.



- ❖ Aquaculture fish, like farmed salmon. Aquacultured fish grow faster or may have improved nutrition (omega fatty acids) This is not necessarily through putting genes in the fish, but more like controlled reproduction, with humans selecting the characteristics to be passed on.



RISKS AND CONCERNS RELATED TO GMOs AND GMFs:

- Expensive projects may not produce enough benefits to justify them
- Environmental Concerns – the GMO may change the natural native biodiversity by reproducing with wild organisms. This is one of the biggest concerns with farmed fish.
- Health concerns – we don't know if there will be long term effects
- How can we be sure that companies are using GMOs and GMFs for the correct purposes?
- Socioeconomic concerns – world hunger is more of a food distribution problem, not a food shortage problem
- Will companies control the crop markets? Where does the farmer fit in?

THE HUMAN GENOME PROJECT

Thousands of international scientists working to identify the entire base sequence of a human, start to finish, published for the first time in 2001.

Major Findings of the Project:

- **All people share a 99.9% identical genome, in other words, 1 base in every 1000 accounts for all the human genetic diversity**
- **There are fewer genes than we thought, about 35 000 rather than 100 000**
- **We make over 100 000 proteins, so each gene might code for 3 proteins, but we're not sure how**

Benefits of the Project:

- Identifying people at risk for diseases
- Designer drugs to suit your genetics

- Better understanding of gene expression
- A way to compare human to other species

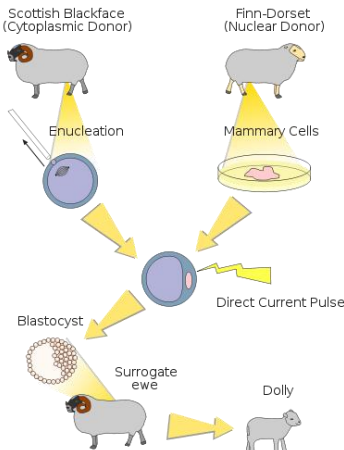
Food for Thought:

- Who should have access to your genetic information? Insurance companies? Employers? Who will be responsible for its security?
- Are you entitled to see the results of testing or benefit from the study?
- Are biotechnology companies entitled to make a profit from publically funded research?
- How can you patent DNA?



CLONING

Clones are genetically identical organism. The first animal ever cloned was a sheep named Dolly in 1997, in Scotland.



In 2001 the first human cells were cloned, researchers were able to make an early blastula without sperm.

There are two kinds of cloning:

- 1) **Therapeutic cloning** to make cells for medical procedures
- 2) **Reproductive cloning** which could theoretically make copies of humans

Possible Benefits:

- Eliminating a wide variety of diseases
- Through therapeutic cloning organs may be made, this would reduce wait time for organ transplants

Concerns:

- Debate over our role in nature – should we manipulate just because we can?
- Use and destruction of embryos
- Are we playing “God”?
- What purposes would cloning whole humans serve?
- How are cloned organisms different from originals? Do they age prematurely? (Dolly died very young) Are there higher mortality or deformity rates?

