

# Genetic Engineering :

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1977 - Frederick Sanger

- discovered the complete base sequence for one type of virus, identified all 9 of its genes, first to do so...opening a new world for genetic procedures and study

Now, **genetic engineering** (the changing of an organisms genome to serve some purpose) is still based on **three** important early discoveries...

We needed...

- a way to break DNA at certain specific chosen spots, and not just randomly ( the use of **restrictive endonucleases**)
- a process to copy DNA over and over again in the lab, ( called “**amplifying**” the DNA sample)  
( using bacteria carriers called “**vectors**” )  
And  
( **polymerase chain reaction**, or “**PCR**” method )
- a way of sorting different size DNA molecules  
( **gel electrophoresis** )

## Restrictive Endonucleases :

Are enzymes made by prokaryotic cells. Many types. Each type recognizes a certain base sequence of DNA and cuts at that specific point...called a “**restriction site**”.

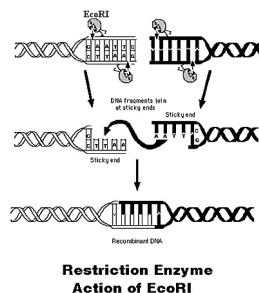
2 reasons why these enzymes are useful...

- we know exactly where they are going to cut DNA, and they cut the same way all the time....making smaller evenly sized DNA pieces called “**restriction fragments**”.
- they cut the DNA in a way that leaves a few unpaired bases sticking off ( called a “**sticky end**” ), that we can use to join pieces of DNA back together in new ways ( called “**recombinant DNA technology**”, making recombinant DNA, or rDNA )

## DNA Amplification :

The process of making a large sample of sample. Accurate copies are made over ways this is done...

Using a “**Vector**”... bacteria are sample for



DNA from a very small and over again. Two

used to multiply the you.

Treat the bacteria with the same restrictive endonuclease used to make the fragment you need copied. This cuts their plasmid in complementary places

The sticky ends are complementary, so your fragment will become part of some of the bacteria cells as part of their DNA

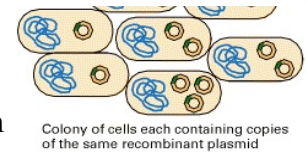
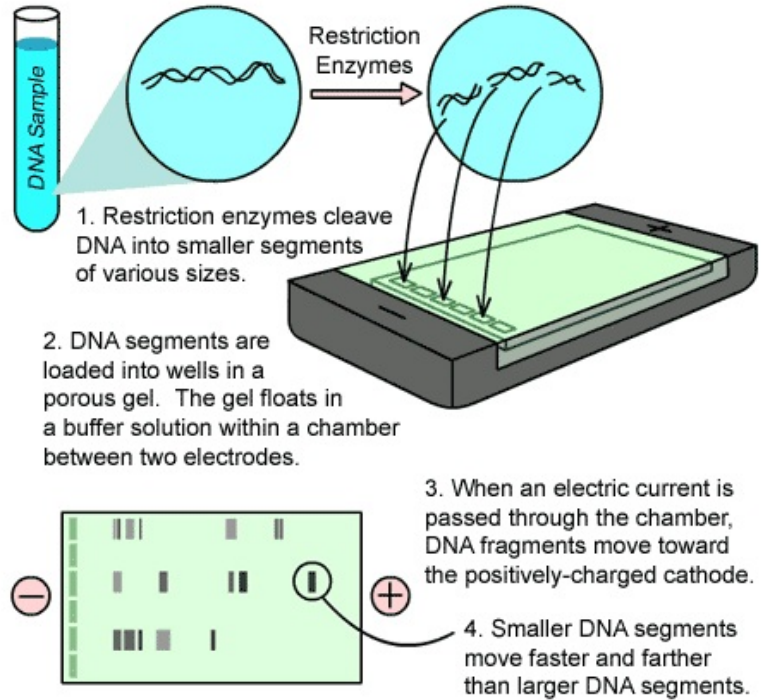
plasmid

Separate the cells you want from those you don't

Let the recombinant cells do their cell cycle division thing.....let them make millions.

Works OK for larger pieces of DNA, but there's a faster way of making smaller pieces....an automated way...

Figure S-2: Gel Electrophoresis



**Using Polymerase Chain**

**Reaction method, or PCR :** (1986 Nobel prize to Kary Mullis)

Your tiny DNA fragment sample is put in a solution with lots of nucleotides and DNA primers. Its heated. Your sample unzips. Its cooled. 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, again to get 8, 16, 32.....if your sample contains several thousand fragments, you can make billions real fast.

Gel Electrophoresis :

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

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

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

Add electricity, and the fragments separate to make a distinctive DNA fingerprint pattern of bands in the gel.

How do we use all this stuff ?

- Crime scene investigations..... single hair follicles, blood, semen, epithelial ( skin ) cells, sweat...anything that provides even the smallest sample can be predictably cut up, copied, and analyzed.
- 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... Who's your daddy ? Compare DNA fingerprints. DNA doesn't lie.
- Genetically modifying plants and animals ( crop growth, artificial hormones, PCB and oil eating bacteria, plants that glow near land mines !)

## **Genetically Modified Organisms ( GMO's) and Genetically Modified Foods ( GMF's ) :**

- 1990 - somatotropin hormone in cows copied using bacterial vectors, commercial production, injected into developing cows, they grow big, make more milk.  
  
1994 this engineered cow "BST" treatment approved in the U.S. Debate over how safe everything is still rages. Monsanto Chemical Corp ( Canada) has been trying for years to get this hormone treatment approved in Canada, but no doing.

### **Other examples....**

- over 50 GM plant species approved and growing in Canada **already**, including over 50 % of corn and canola crops  
  
Herbicide resistant corn...  
take herbicide resistant gene from some bacteria, put it into corn gametes, make herbicide resistant corn...you can spray without damaging crops.  
  
Making artificial human hormones...  
Using rBacteria to make huge volumes of human insulin for commercial sale, lowering the cost of treatment and eliminating allergy risk with animal sources.  
  
Bioremediation...  
Using modified creatures for environmental clean up

PCB Eating Bacteria...

gene for enzyme decomposing highly toxic PCB's inserted into bacteria that can live in soil. They decompose PCB's in contaminated sites with less expense and do a better job.

Oil Eating bacteria...

Same idea, clean up every nook and cranny on a molecular level. Oil spill recovery.

Filtering smoke stack emissions

Filtering water on a molecular level

“Golden rice” -

fortified with extra beta carotene and iron production, distributed in third world countries for better nutrition with less effort and land.

Aquaculture fish that grow faster, or have improved nutrition ( omega fatty acids )

Transgenic salmon have been modified, one kind makes an antifreeze so they can be raised in colder aquaculture sites, another grows up to 10 times faster...ready for market a year earlier. Easier business start up and more product in less time. Supporters say its just artificial selection made faster, with no new genes made, just moving stuff from fish to fish, so there's no health risk, we could help world hunger or restore sagging wild populations. Opponents say we don't know enough about how the gene might become part of the fish, or its long term effects in fish consumers.

## **Risks and Concerns Related to GMO's and GMF's :**

Expensive projects might not produce enough of a benefit to justify them.

Proposals for projects in Canada go to Health Canada and the Canadian Food Inspection Agency. They consider the potential costs and benefits, the risks of carrying toxins or allergens, the soundness of the scientific processes involved, and how the new organism is different from the original.

Consumer groups opposed to this research identify three areas of concern...

Environmental concerns - the GMO might change the natural native biodiversity of Canada by reproducing with wild organisms

Herbicide resistance might encourage more herbicide use, and more related environmental problems, or create new plants or insects resistant to our control measures

Health concerns - we don't know much about the long term effects of GMO's and GMF's

How can we be sure that users use GMO's and GMF's according to regulations, or for what purposes ?

Socioeconomic concerns - world hunger is a food distribution problem, not a food shortage problem, and these expensive projects don't fix that.

Will food supplies become the property of big corporations ? What happens to small scale rural Canadians ? Will patent holding companies own the lions share of the crop markets ?

## Bringing DNA Technology to the Human Animal :

### The Human Genome Project...(HGP)

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 ?

- all people share a 99.9 % identical genome....in other words, 1 base in every 1000 accounts for all the human genetic diversity
- there's fewer genes than we thought....35,000 instead of about 100,000
- we make over 100,000 proteins, so each gene might code for 3 proteins ( not sure how though )

Where might it take us ?

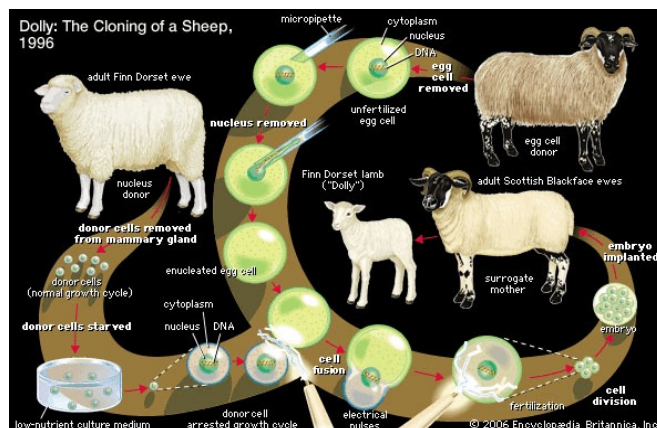
- identifying people at risk for diseases before they have the disease
- designer drugs suited to your genetics
- better understanding of gene expression
- comparison to other species, new classifications

Related issues ?

- who should / should not have access to your genetic information ? Insurance companies ? Employers ? Who owns it and is responsible for its security ?
- are you entitled to see results of testing, or any benefit from its study ?  
Read the NFLD and LAB example on pages 619-620 !
- are biotechnology companies entitled to make a profit off of publicly funded research ? If they don't make a profit, what keeps them in business ?

## Cloning Animals :

Clones are genetically identical organisms. Early experiments "proved" that adult differentiated cells couldn't be used for cloning purposes, only undifferentiated cells. We know that's not true now. The first animal ever cloned using adult donor cells was a sheep named "Dolly" in 1997, in Scotland.



## What about Cloning Humans ?

2001 - first human cells cloned, able to make an early blastula without sperm

2 kinds of cloning...

Therapeutic cloning makes cells for medical procedures

Reproductive cloning would theoretically make copies of humans

<http://www.religioustolerance.org/cloning.htm>

Possible benefits ?

- eliminating a wide variety of diseases
- organs for transplant, greatly reduced wait lists

Concerns ?

- debate over our role in this environment ( are we a part of it, or masters of it ?) And our attitude towards other creatures....are they exploited to suit our needs ? Should we manipulate the natural workings of things for our own benefit...just because we can ?
- the use and destruction of embryos
- its not our place to play "God"
- what purposes would human cloning serve ?
- how are cloned organisms different from the originals...organs / animals age prematurely ? Higher mortality or deformity rates ?