SECTIONS OF GENE TECHNOLOGY - order to be read

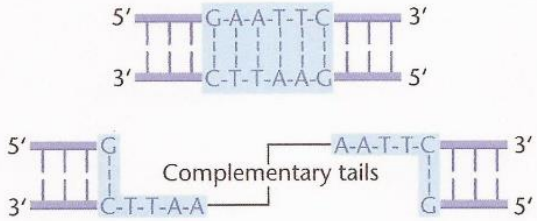
1. Obtaining a gene – restriction enzymes and reverse transcriptase (Mills and McAllister)
2. DNA probes and electrophoresis (Lee and Kerr)
3. Gene transfer – getting a gene into a plasmid (Cooke and Russell)
4. Gene transfer – getting the plasmid taken up (Milliken and Palmer)
5. Genetically Engineered Microorganisms, transgenic plants (not case study) and transgenic animals (Blackmore and Kennedy)
6. Gene therapy (not case study) (Burrows and Connolly)
7. Genome sequencing (before case study) (Withers and Clarke)
8. Genetic mapping (after case study) and knockin knockout technology (Morrison and Stewart)
9. Ethics of gene technology (before case study) Ethics of gene technology (after case study) (Paul and French)

Restriction Endonucleases

-Can be used in obtaining a desired gene for transfer

-Unlike other enzymes, it doesn't bind to a specific molecule but a base sequence called a recognition sequence. Eg Eco R1 has a recognition sequence of GAATTC.

- Can produce a staggered ends known as sticky ends. They occur when an enzyme eg EcoR1, makes a cut between adjacent bases when do not lie directly across from each other.



-Blunt ends, the alternative cut made by an enzyme, that is made when the enzyme cuts between adjacent bases directly across the DNA molecules. Eg Hae111 which recognitions sequence is GGCC,

Reverse Transcriptase

-This is another type of enzyme involved in gene technology

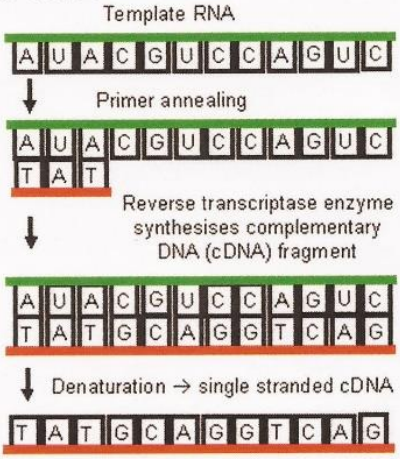
- It is found in nature in retroviruses such as HIV

- It is used to make a single strand of DNA (cDNA) using the mRNA as a template.

- mRNA of desired gene can be found by doing an "advanced search" ie. Looking in a region where the desired gene is active

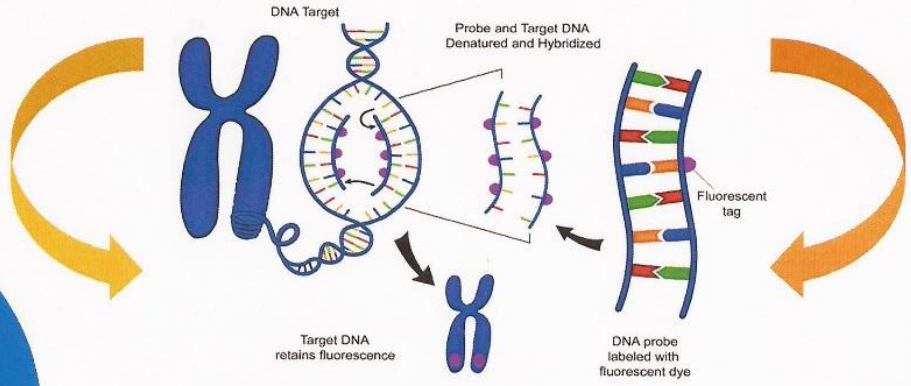
- After a single strand of DNA is made, DNA polymerase catalyses the reaction to add free nucleotides to make the DNA double stranded.

-The cDNA can be used as a template for amplification by PCR, or to generate a cDNA library.



DNA PROBES

DNA probes can be used to identify sections of DNA that contain a specific sequence of bases.



NB: Important to wash off any DNA probes that don't attach to their target sections- labelled probes will show up whether they are hybridised or not

NB2: DNA probes will attach to single strands of DNA only. Therefore when using DNA probes it is necessary to treat the DNA so that it is split.

WHAT IS A PROBE?

DNA probes are short single strands of DNA which are complementary to the target section of DNA so that the bases will hybridise to the target section if present.

LABELS

DNA probes are labelled either radioactively or fluorescently for them to be identified. X-ray film is used to detect radioactive labels and UV light is used to detect fluorescent labels.

USING PROBES

Restriction endonucleases hydrolyse the targeted DNA into fragments that are then separated by gel electrophoresis. The DNA is then transferred to a nylon membrane where a DNA probe is added. If the target sequence is present the DNA probe will hybridise with; if not it will then be washed off. UV light or X-ray film is then used to detect the DNA probe.

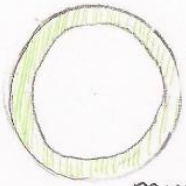
INSERTING THE GENE INTO A VECTOR

human DNA

gene removed with restriction endonuclease. (1)

here plasmids are being used as vectors = "gene delivery tools".

Bacterial Plasmid

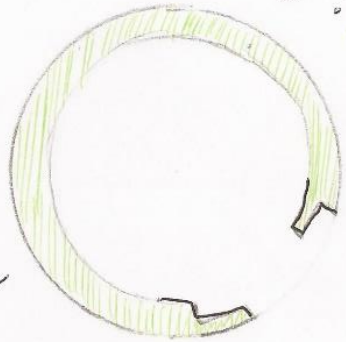


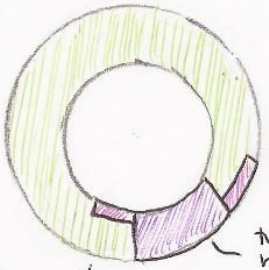
closed bacterial loop of DNA

must be cut to create the ends to which the donor DNA can join to.

∴ the plasmid is cut using (2) the same endonuclease as the donor was cut with.

= complementary sticky ends.





This enzyme joins the DNA backbones by phosphodiester bonds between sugar phosphate backbones.

once paired the human gene is annealed into the bacterial plasmid by DNA LIGASE (3)

this causes a perfect join of matching bases

Therefore can be seamlessly spliced into plasmid.

Plasmid taken up by bacteria.

The end result

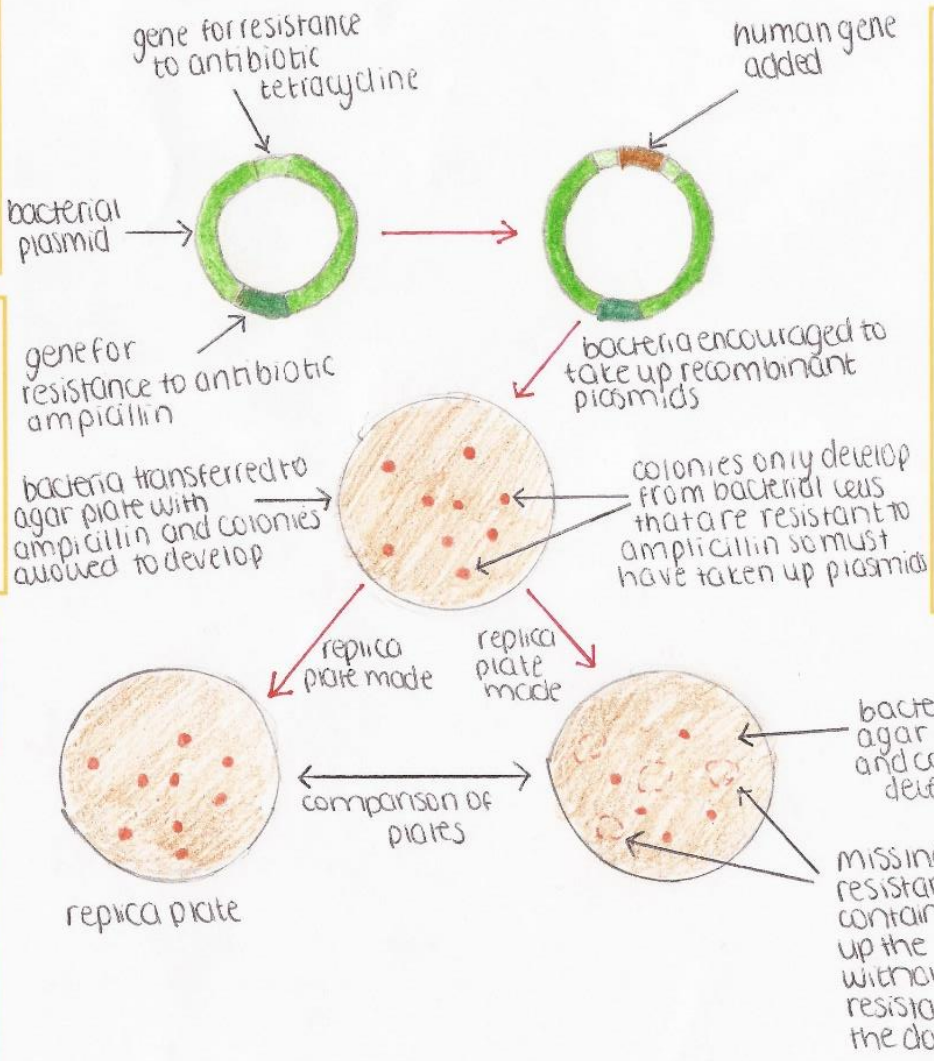
(4) now plasmid DNA = recombinant DNA as it contains foreign DNA.

Gene Technology: inserting the gene into the host cell – the role of the plasmids

After adding the donor DNA into the plasmid vector, the next step is to encourage the host cell to take up the plasmid. Usually in recombinant DNA technology the host cell is a bacterial cell that can be cloned and then used as "factories" to produce the desired product.

If the bacterial cells are incubated with calcium ions and subjected to heat shock there is a greater chance that they will take up the recombinant plasmids. The key thing following inducement of plasmid uptake is to identify the transformed bacteria.

Using marking genes that can be identified is a common technique used to identify recombinant or transformed bacteria. Bacterial plasmids almost always contain the gene involved in the development of antibiotic resistance in the bacteria and some plasmids have genes that are resistant to two or more antibodies. One example is the R-plasmid which has the genes for resistance to the antibodies tetracycline and ampicillin.



A restriction enzyme is used when cutting R-plasmids before inserting donor DNA. This enzyme cuts the bacterial plasmid within one of the genes that provides antibiotic resistance. This is significant as if the donor DNA anneals into the plasmid, the plasmid will no longer have a gene that confers resistance to tetracycline. Although the gene that confers resistance to ampicillin is unaffected.

Gene Technology

Inserting the gene (desired piece of DNA) into the host cell - the role of plasmids.

Replica Plating:

- involves 'blotting' of the original plate with an absorbent pad
- then pressing this against the surface of a fresh plate
- this allows some of the cells of each colony to be transferred
- colonies will form (or not) on equivalent positions on the new plate

N.B: Other markers are now available - a gene that produces a protein that fluoresces in certain conditions can be incorporated into a plasmid.
When tested, host cells that have taken up the donor DNA WILL NOT be able to fluoresce

Using DNA Probes:

- Used to identify if host DNA is recombinant
- Once this is confirmed, the next phase is to clone bacteria - ensures large numbers are produced
- typically grown in large fermenters where conditions are ideal for rapid growth and production of the desired product.
- transformed bacteria can be described as genetically modified organisms (GMOs) or genetically engineered microorganisms (GEMs)

Genetically Modified Organisms (GMOs)

Textbook Pages: 203,4+7

By Rachel Blackmore and Rachel Kennedy

Genetically Engineered Microorganisms (GEMs)

Bacteria are ideal for modification as:
- it is relatively straightforward to insert donor DNA (aided by plasmids)
- more accepted use (less ethical issues then plants + animals).

Therefore: Scientific research + commercial development in this area is at a more advanced stage.

INSULIN

- Advantages of this method include being able to produce sufficient quantities for increasing demand, identical hormone produced (due to use of the 'human' insulin gene).
- Insulin used to be extracted from dead domestic animals which was more time consuming + ineffective in comparison. Also this non-human insulin is slightly different to that of humans and this could cause allergic reactions.

Other products produced by GEMs: human growth hormone, enzymes, adhesives, lung surfactant protein + interferon (drug used in cancer treatment).

Transgenic Plants

Transgenic crop plants have enormous potential benefits for commercial and health use. Most are commonly known as **Genetically Modified (GM) Crops**.
Methods to insert donor DNA:

- ***Agrobacterium tumefaciens*** – Genetic engineering allows the gall-forming genes to be removed from the bacterium + be replaced by donor DNA. Normally, this soil bacterium causes the growth of tumour-like galls in the plants it infects. By entering a damaged area of the plant, integrating bacterial DNA into the plant's + causing rapid cell growth => galls.
- **'Gene guns'** can also be used to insert the donor DNA into the host cells. Microscopic pellets are coated with donor DNA so they can be fired (using compressed air) into the host cell – this is a bit 'hit or miss' as to whether the donor DNA will actually become incorporated. Although it is a method that can be used if the species is resistant to Agrobacterium.

NOTE: Donor DNA should be introduced to a small no. of cells in a tissue culture (so that all the mature plant calls will contain the recombinant DNA).

Transgenic animals

Techniques for inserting donor DNA into animal cells:

- Encapsulating donor DNA in liposomes- has the advantage of using lipids which makes it easier for DNA to cross the lipid bilayer of the cell surface membrane.
- Electroporation- involves disrupting the cell membrane, making it more permeable by the use of high voltage treatment.
- Viruses can be used to insert the donor DNA into animal cells.

The donor genes must be inserted directly into the fertilised eggs of the animal at the one-cell stage to ensure that the donor gene (***transgene***) will be present in all the daughter cells of the developing organism.

Transgenic organism	Genetic modification and consequence
Chicken	Human gene for production of antibody mi-R24 inserted. Antibody obtained from eggs and used in skin cancer treatment.
Cow	Human gene added to code for alpha-lactalbumin - gives milk more similar composition to human milk. Other genes added to increase milk production or milk with reduced lactose content.
Sheep	Human gene coding for blood clotting factor (Factor VIII). Purified from ewe's milk + used to treat haemophilia. Can also produce serum protein alpha-1-antitrpsin which is important in regulating activity of proteases and used in treatment of cystic fibrosis.
Goat	Human gene added to produce anticoagulant anti-thrombin. Can be given to individuals deficient in anti-thrombin due to defective allele.

Gene Therapy

DEFINITION: The introduction of normal genes into cells in place of missing or defective ones in order to correct genetic disorders.

There are two distinct approaches to gene therapy:

1. Somatic-cell gene therapy

The gene therapy only targets the affected tissues. This technique can be used when a condition caused by defective gene(s) affects specific, and easily reached, parts of the body. Somatic-cell gene therapy can be used at any stage of the individual's life.

2. Germ-line gene therapy

This involves replacing the defective gene(s) in the fertilised egg. In germ-line gene therapy, all the cells of the developing individual are normal and there is no issue with targeting affected tissues in the body, which is a problem with somatic-cell gene therapy. With germ line gene therapy the defective gene will not pass on to the future offspring as the gametes produced will not contain the defective gene.

Currently it is only somatic- cell gene therapy that is used. Germ- line gene therapy is beyond current technological capability and raises so many moral and ethical issues that it is presently not permitted.

Getting the functional allele into the recipient cells

Viruses are used as one type of vector in gene transfer.

- **Adenoviruses** are viruses that cause respiratory infections. They are ideal for gene therapy involving the lung tissue as adenoviruses are able to inject their DNA into lung epithelial cells. Before adenoviruses can be used in therapy they have to have their 'harmful' disease causing genes removed as well as the donor DNA being spliced in. It is difficult to remove the disease-causing genes without dismantling the genes that allow the virus to enter cells and insert their genetic material. They may cause infection. Some individuals may be immune to adenoviruses and they are unable to penetrate the respiratory cells.

- **Retroviruses** can be used to target host cells. When the host cells are affected by the retrovirus, it's RNA and any additional donor RNA is converted by reverse transcriptase into DNA in the host cell. If the donor DNA replaces or supplements the defective DNA in the host cell, then gene therapy is possible.

- **Liposomes** are artificial lipid vesicles containing donor DNA. The lipid coating of the vesicle protects against degradation and helps the donor DNA bind to the appropriate target cells. This process is aided by using specific marker cells, e.g. monoclonal antibodies, that help the liposome target the appropriate recipient cells. Having a lipid coat means that the liposome can pass relatively easily through the lipid part of the phospholipid cell-surface membrane.

By Lucy and Sarah

When the donor DNA is in the appropriate host cell it can function in two distinct ways;

The donor can become incorporated into the host DNA

It can function as independent DNA units (episomes) in the nucleus that are not incorporated into the main nuclear DNA

Although still at the early stages of its development, gene therapy has huge potential in replacing (or supplementing as with episomes) defective DNA that causes many of the major genetic diseases today. Major difficulties still surround the ability;

- X Targeting all the affected areas of the body for a particular condition.
- X The treatment is often short-lived as any new and replacement cells will not have the donor DNA.
- X Offspring of treated individuals may still be affected, depending on the genetics involved.
- X The defensive strategies employed by cells may destroy the introduced DNA or prevent it being 'switched on'.
- X Many genetic disorders (or disorders with genetic predisposition) involve many genes across a range of chromosomes, e.g. Type 1 diabetes, it can be very difficult to both identify the genes involved and effectively incorporate all the donor DNA necessary into host cells.

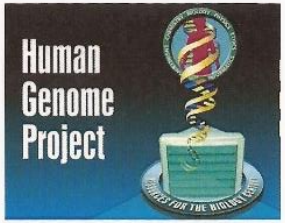
Gene therapy is used to treat a range of disorders and many more are coming on-stream. It is a fast changing area of gene technology with both huge medical potential and almost certainly complex underlying ethical issues, particularly as the boundaries between addressing genetic malfunction and genetic enhancement become blurred. The potential of gene therapy can only be realised when there is excellent knowledge of the total DNA complement with all relevant genes being effectively mapped.

By Lucy and Sarah

Genome sequencing

The complete DNA (base or nucleotide) sequence is its genome. The genome is a "map" of the DNA in an organism. The genome includes **all the DNA**, i.e. exons and introns.

Genome sequencing is working out the base sequence in an organism i.e. the order of bases along each chromosome.



Key steps in genome sequencing:

In the early stages of genome sequencing, scientists targeted more simple organisms. Key stages of early sequencing included:

- Yeast was the first eukaryotic organism to be fully sequenced (has approx. 16,000 genes and 16 chromosomes)
- The roundworm (nematode) was the 1st multicellular organism to be sequenced (has 20,000 genes and 6 chromosomes)
- Other organisms sequenced include: virus phage λ, the bacterium *Escherichia coli*, the plant *Arabidopsis thaliana* and the fruit fly *Drosophila melanogaster*

The Human Genome Project:

The Human Genome Project (1990-2003) was a massive multinational project that aimed to work out the DNA nucleotide sequence in the human genome.

The human genome is just over 3 billion base pairs in length and contains around 21,000 genes. Only 2% of this DNA codes for polypeptides with the remainder having a regulatory role acting as gene switches or having no known function ("junk" DNA)

Genetic mapping:

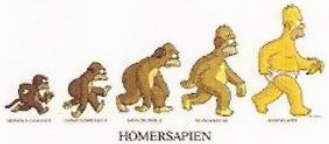
Genetic mapping is identifying exactly where particular genes are on chromosomes (without necessarily knowing the base sequence within the genes.

This is important in locating **defective** genes and in the identification of useful genes that could be used in **recombinant DNA technology**.

Kara and Christine ☺

Applications of genome sequencing:

- Use in DNA fingerprinting in forensics
- The analysis of ancestral relationships between organisms



Benefits of the Human Genome Project and the creation of 'genome libraries'

- More detailed **mapping of genes**- aided in diagnosis and treatment of genetic disorders. Specific genes can be identified using DNA probes. **DNA chips** are DNA sequences that act as probes by being complementary to the nucleotide sequences linked to genetic disease. Can identify defective allele and identify if someone is a carrier of a condition.
- Development of genome sequencing increases the potential of **gene therapy**. Understanding of difference between functional and defective genes (base pairs) helps identify exactly what donor DNA is required/ possibility of artificial donor DNA in 'gene machines'.
- **Designer drugs** can be matched to the genomes of individuals so drug treatments can be more effective and modified to reduce side effects e.g. linking specific treatment for some breast cancer patients to the actual gene that is defective. However, it could be uneconomical to produce personalised medicines despite their medical benefits. Knowledge of the genomes of bacteria/viruses enables antibiotics and vaccines to be developed that specifically target these strains.
- **DNA (genetic) fingerprinting** is dependent on the differences in repeat DNA sequences- (MRSs) or short tandem repeats (STRs). Individuals have different numbers of repeat sequences that occur in introns and form part of the 'junk' DNA.
- **The primary structure of proteins** can be worked out by knowledge of gene sequence e.g. If DNA sequence has been mapped to show that a particular gene lies between two specific points, then knowledge of the bases between those points will allow the mRNA codons and amino acid sequence to be determined. Molecular modelling software can predict 2°, 3° and 4° structure based on predicted bonding and folding arrangements.
- Greater understanding of **ancestral links** between organisms and their **evolutionary development** – it can confirm or modify these relationships. Before arrival of genome sequencing similarities in morphology, anatomy, biochemistry and even ecology have been used to work out ancestral relationships.

Genetic Mapping + Knockin/Knockout Technology

By Katie + Claire

A gene knockin

is where a paticular gene has been added.

It can be used to deliberately add a defective gene to study disease progression

Genetic Mapping

Definition:

Genetic mapping identifies exactly where paticular genes are on chromosomes.

The International Mouse Consortium was established in 2007 with the aim of making data available from the knockout of all protein-coding genes to the medical and scientific communities

Why is it useful?

- Finds the location of defective genes
- Identifies useful genes that can be used in recombinant DNA technology.
- Aids in the diagnosis and treatment of genetic disorders.

A gene knockout

is a transgenic organism in which a gene has been removed or made inoperative.

Most Vertebrate knockout technology has involved the mouse as it:

- Is biochemically + physiologically similar to humans
- It has a short life cycle
- Can be easily kept in laboratory conditions
- People are not as ethically opposed to using mice than for larger mammals.

Why is it useful?

With gene knockouts, the effect of loss of gene function can be modelled in non-human organisms.

Helps with the progress of the understanding of genetic diseases such as:

- Cystic Fibrosis
- Muscular Dystrophy
- Huntington's Disease

ETHICS

1. Pre-implantation Genetic Diagnosis (PGD)

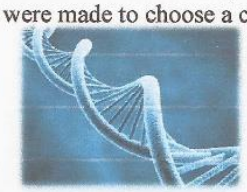
What is PGD?

A method of screening embryos (which are conceived by IVF) for genetic abnormalities. Only 'healthy' embryos are implanted in the uterus.

What ethical questions does PGD raise?

- Is it ethical to destroy embryos because of genetic abnormalities?
- Might this technology lead to **eugenics* or** 'designer' children?
- Will it allow sex-selection? (A recent international survey found that 2% of more than 27,000 uses of preimplantation diagnosis were made to choose a child's sex.)

2. Gene Therapy (GT)



What is GT?

The action of inserting a functional allele into cells affected by a genetic condition in an attempt to get the cell to function normally.

What ethical questions does GT raise?

- It is very expensive. Would the money be better used in reduce hospital waiting lists or to buy vaccines or antibiotics for those in developing countries? Which will save more lives?
- Is the risk of introducing viruses and donor DNA into the cell too great?
  - E.g. In the French trial of gene therapy for SCID (severe combined immunodeficiency) in 2002, two of eleven babies in the clinical trial died from leukaemia, the cause of which was assumed to be the disruption to host DNA.

3. Personalised Medicine and Biobanks (PM and Bb)

What are PM and Bb?

PM: Very specific drug treatments tailored to the genome of an individual or even the genome of specific cancer tumours caused by the presence of specific genes.

Bb: A way of storing the genetic data for some or all of the population. It is a stepping stone towards PM.

What ethical questions are raised by PM and Bb?

- PM is very expensive. Again, would the money be better used elsewhere?
- Should gathering of data be mandatory?
  - If Yes, there are consent issues.
  - If No, it is likely people who are from ethnic minorities or are less educated will be underrepresented.
  - With both there are issues with data safety.
- How practical is PM, especially in dealing with complex issues with multiple genes contributing to the pre-disposition or when environmental influences are more important?
  - Most would say that diet, obesity and lack of exercise are the major factors in contributing to coronary heart disease with genetics playing a smaller role.
- Even 'straight-forward' genetic diseases can be complex.
  - E.g. cystic fibrosis: 70% of sufferers have the same mutation, but the other 30% of cases are caused by up to a thousand different mutations

***Eugenics:** a social philosophy advocating the improvement of human genetic traits through the promotion of higher reproduction of people with desired traits (positive **eugenics**), and reduced reproduction of people with less-desired or undesired traits (negative **eugenics**).

Ethical Imperative

People have few ethical issues with GMO (genetically modified organisms). However there are strict regulations concerning the containment of transgenic microorganisms with considerable resources used to prevent their escape into the wild.

Containment measures are:

• *Licenced and purpose built laboratories with air filters to suck air back into the lab.*
• *Tightly controlled procedures for staff in terms of access and cleaning*
• *Special disinfectant procedures*
• *Poorly adapted bacteria stains to survive outside controlled lab conditions and poorly equipped in humans.*
• *'Suicide genes' in these strains that are activated if conditions don't remain within specific pH or temperature limits.*

No matter how good these measures are there is a possibility that GEMs can escape and survive beyond the lab.

Reasons why GM Crops are in public consciousness are that they're more visible or people have to consider eating GM crops that has helped created an opposition. We know that 'Superweeds' can be created due to their pollen escaping but 'superweeds' but it can also be created by natural selection. This means GM technology is no more likely to cause development of 'superweed' than could occur naturally. Free trials with GM crops are held in the open and not closed.

Producing disease resistant, more nutritious and more productive crops – do the benefits outweight the disadvantage? Particularly when there is a shortage of food people will starve as they cant grow or buy food. This problem only gets worse as human population grows out of control. Climate change makes land unsuitable and pesticide resistance increases.

Genetic screening

Able to screen before or after birth for conditions such as Down Syndrome. What if it's a positive result – Bring up child with medical disability or destroy the foetus? – a difficult choice for many.

Genetic screening after birth has fewer ethical implications. Phenylketonuria (PKU) is a disease where individuals lack an enzyme leading to severe mental handicap. All new-born children are tested for the presence of this disease, so if they are positive a diet low in phenylalanine is give so PKU doesn't develop.