

GENE TECHNOLOGY

Key Objectives

- To understand how the human insulin gene can be transferred to bacteria in such a way that the bacteria become capable of synthesising human insulin
- To be able to explain the need for promoters to be transferred along with genes during gene technology
- To be able to explain the role of fluorescent markers in gene technology – and why these are now used in preference to antibiotic resistance genes
- To be able to describe the benefits and hazards of gene technology (with reference to specific examples) and discuss the social and ethical implications
- To be able to outline the principles of electrophoresis (as used in genetic fingerprinting and DNA sequencing)
- To be able to describe the causes and outline the symptoms of Cystic Fibrosis (as an example of a recessive genetic condition)
- To understand the extent to which progress has been made towards successful gene therapy for Cystic Fibrosis
- To be able to discuss the role of genetic screening for genetic conditions and the need for genetic counselling

Key Definitions

- *Gene technology* – this term really covers techniques such as genetic engineering, the creation of genomic libraries of DNA and DNA fingerprinting.
- *Genetic engineering* – the transfer of a gene from one organism (the donor) to another (the recipient) e.g. the genes coding for human insulin, growth hormone or the blood clotting factor, Factor VIII may be removed from human cells and transferred to bacteria.
- *Promoter* – a length of DNA (usually about 40 bases long) situated next to genes and which identify the point at which transcription should begin.
- *Marker* – a gene which is deliberately transferred along with the required gene during the process of genetic engineering. It is easily recognised and used to identify those cells to which the gene has been successfully transferred.
- *Genetic fingerprinting* – the analysis of DNA in order to identify the individual from which the DNA was taken to establish the genetic relatedness of individuals. It is now commonly used in forensic science (for example to

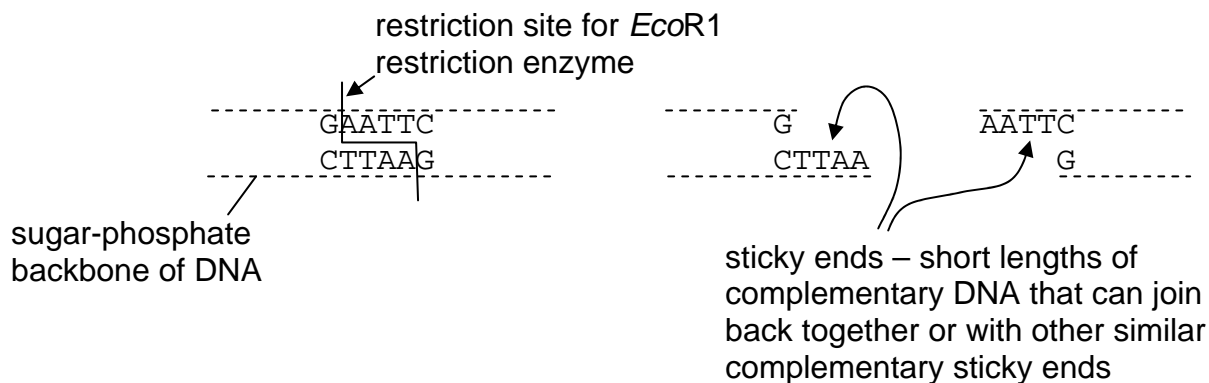
identify someone from a blood sample) and to determine whether individuals of endangered species in captivity have been bred or captured from the wild.

- *DNA sequencing* - the determination of the precise sequence of nucleotides in a sample of DNA or even a whole genome e.g. the Human Genome Project.

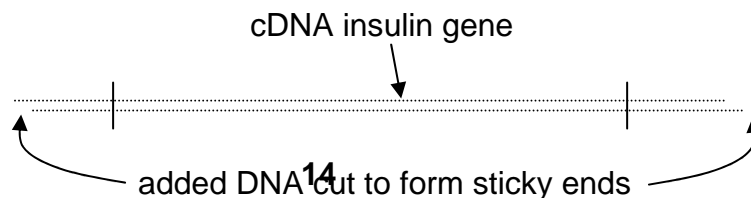
Key Ideas

Steps involved in the genetic engineering of bacteria to synthesise human insulin

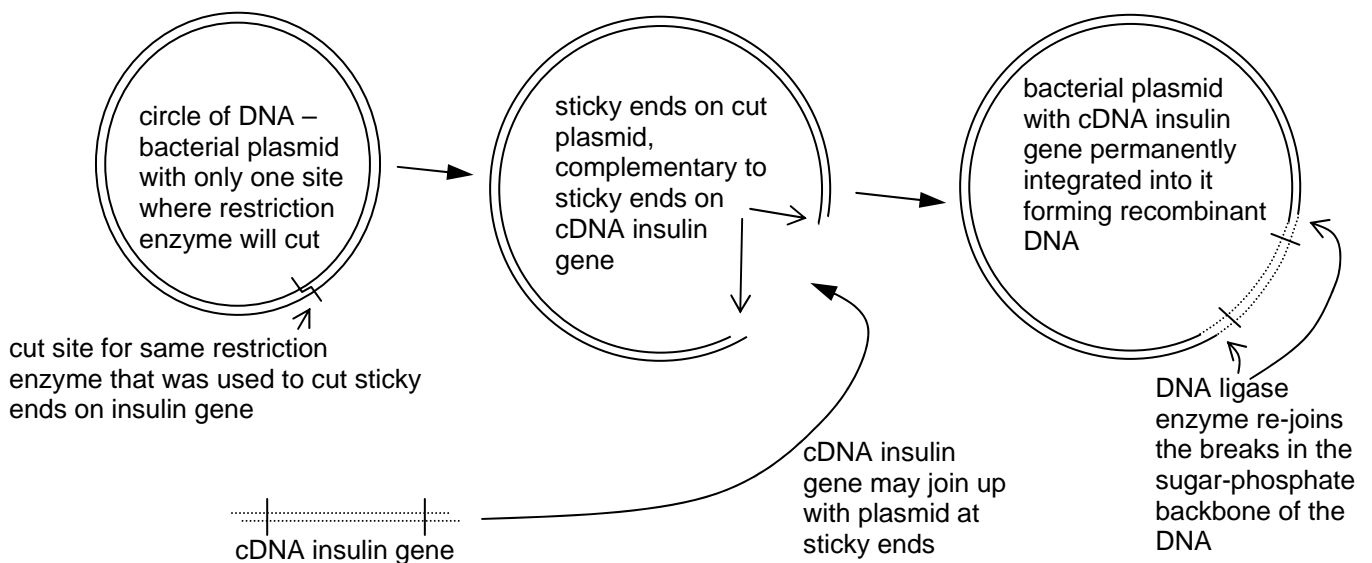
- 1 Human insulin gene must be identified. There are various ways in which this might have been done, described at the end of this section. What was actually done, in the late 1970's, was as follows:
 - Insulin-producing cells from human pancreas tissue synthesise large amounts of the protein, insulin, for which they make large amounts of mRNA. This mRNA has a genetic code complementary to the key portions (exons) of the human insulin gene. Some of this mRNA was isolated from such cells.
 - The mRNA was incubated with a mixture of free DNA nucleotides and reverse transcriptase (an enzyme from viruses that use RNA as their genetic material). This produced a single strand of DNA known as complementary DNA or cDNA, which is a copy of the informational strand of the human insulin gene.
 - The single strand of cDNA was then made double stranded using DNA polymerase, and cloned to make many cDNA molecules using the polymerase chain reaction (PCR)
- 2 Additional, non-coding DNA was added to the ends of the cDNA insulin genes so that 'sticky ends' could be produced using *restriction enzymes* (also called restriction endonucleases). Restriction enzymes cut DNA at specific base-sequences – their restriction site, for example, *EcoR1* cuts at the sequence GAATTC. Some restriction enzymes leave 'sticky ends' (short lengths of unpaired bases at each cut) as shown below.



The restriction enzyme was chosen so that it would not cut the insulin genes into pieces, and would leave sticky ends at either end of the gene, shown below.



- 3 The gene is then transferred to a bacterial plasmid - a small, circular DNA molecule found in bacteria and separate from the bacteria's main DNA molecule. The gene was inserted into a selected plasmid by cutting open the plasmid using the same restriction enzyme that was used to make sticky ends at either end of the cDNA human insulin genes – again, leaving complementary sticky ends. If the insulin genes and the cut plasmids are mixed, the complementary bases in the sticky ends will pair up. This may join the gene into the plasmid. (Unfortunately some plasmids rejoin without gaining the desired gene.) Ligase enzyme is used to re-join the breaks in the sugar-phosphate backbone of the DNA so that the gene is permanently added to the plasmid, forming recombinant DNA.



- 5 The plasmids containing the human insulin gene are then transferred to bacterial cells. This is brought about by mixing the plasmids with bacteria, some of which will take up the plasmids. The bacteria which take up plasmids containing the human gene are said to have been *transformed*. The transformed bacteria are then cloned to produce large numbers of genetically identical offspring, each containing the recombinant plasmid, and grown on a large scale. Every time a bacterium divides, it will replicate the human insulin gene. In each bacterium the gene will be expressed, being transcribed and translated in the bacterium to produce human insulin.
- 7 The bacterium *Escherichia coli* has been transformed in this way and has been used since 1982 to produce human insulin.
- 8 The steps above are a simplification of the process used to manufacture human insulin using recombinant DNA. This is partly because it has been done several times, improving the process each time it has been done as we understand more of the genetic mechanisms involved.

Human insulin is a small protein which does not contain the amino acid methionine, but does have quite a complex structure, with two polypeptide

chains, A and B, joined to one another by covalent disulphide bonds. The presence of two chains means that it has a quaternary structure, and also that two separate genes are used, one to make each polypeptide. In order to produce each of these two polypeptide chains separately, the two genes were added into the lac operon (see below, in the section on promoters) of the B-galactosidase enzyme of *E. coli*. Before the start of the cDNA code for each of the insulin genes was inserted an extra triplet, ATG. Look in a DNA dictionary (e.g. at <http://users.rcn.com/jkimball.ma.ultranet/BiologyPages/C/Codons.html>) to confirm that this is the DNA triplet code for methionine.

To make sure that transcription stopped at the correct place, two consecutive stop codes were added at the end of the cDNA for the A chain, and also the cDNA for the B chain. In each case the triplet TAA was followed by the triplet TAG. Look these up in a DNA genetic dictionary to confirm that they are stop codes.

This had to be done separately to different plasmids, so that some plasmids contained the gene for the A chain, and others the gene for the B chain. When the genetically engineered *E. coli* containing both types of plasmid was grown in the presence of lactose, the lac operon genes were turned on but instead of producing B-galactosidase, produced some proteins containing the first part of the bacterial protein, followed by methionine and then either the insulin A chain or the insulin B chain. When these proteins had been separated from the bacteria, they were treated with cyanogen bromide, which cuts the amino acid sequence at methionine, separating the insulin chains from the remains of the bacterial protein. When the mixture of A and B chains is treated to promote formation of disulphide bonds, insulin forms.

- 8 The latest methods for manufacturing genetically engineered human insulin use eukaryotic yeast cells rather than prokaryotic bacterial cells. The yeast cells can use eukaryotic promoter sequences and have Golgi bodies, so that they produce insulin that is released already in the correct 3-dimensional conformation to achieve maximum activity in humans.

<http://www.littletree.com.au/dna.htm>

Other methods that could have been used to isolate the insulin gene:

- The amino acid sequence of insulin is known, so a DNA dictionary could have been used, and synthetic DNA with an appropriate base sequence synthesised.
- The DNA base sequence of the insulin gene has been found during the human genome project, so a single-stranded DNA probe, radioactive or fluorescent in UV light, could have been made, complementary to part of the insulin gene. If the DNA from human cells was cut up into fragments using restriction enzymes, denatured into single stands by heating, separated depending on mass using electrophoresis and then treated with the probe, the probe would stick only to the DNA fragments containing the insulin gene, allowing the insulin gene to be isolated from the rest of the DNA.

Such methods have been widely applied to isolate other genes for genetic engineering.

The advantages of treating diabetics with human insulin produced by gene technology

Until bacteria were used to produce human insulin, people with insulin-dependent diabetes were injected with insulin derived from pigs or cattle. Although this type of insulin works in the human body, pig or cow insulin does not have exactly the same primary structure as human insulin, so its amino acids sequence, while similar to human insulin, is not identical.

There are a number of advantages of using the human insulin produced by genetically engineered bacteria:

1. it is chemically identical to the insulin that would have been produced had they not been diabetic, so there is little chance of an immune response
2. because it is an exact fit in the human insulin receptors in human cell surface membranes, it brings about a much more rapid response than pig or cow insulin,
3. like natural human insulin, the duration of the response is much shorter than pig or cattle insulin,
4. it overcomes problems related to the development of a tolerance to insulin from pigs or cattle,
5. it avoids any ethical issues that might arise from the use pig or cattle insulin, for example, religious objections to the use of pig insulin or objections from vegetarians to the use of animal products.

Why promoters need to be transferred along with the desired genes

In the DNA of bacteria and other prokaryotes, base sequences called promoters are situated just before ('upstream' of) each gene. These identify the point at which transcription should begin. Usually, these consist of two short six base sequences, TATAAT, situated about 10 bases before the gene and, TTGACA, situated about 35 bases before the gene. The presence of at least one of these is usually necessary to initiate transcription of the gene in prokaryotes.

In the case of insulin, the first successful recombinant DNA involved using the promoter of an existing non-essential gene, for an enzyme involved in lactose metabolism (B-galactosidase). The human insulin gene was inserted into the existing gene. The promoter for this gene remained intact. There is also a lactose-sensitive regulatory sequence that is designed to turn on the natural B-galactosidase in the presence of lactose. The promoter, regulator and gene, are together called an operon, in this case the lac-operon. The effect of all this is that when the genetically engineered E coli, containing the human insulin gene in its lac-operon, was exposed to lactose, it transcribed a polypeptide that contained the first part of the B-galactosidase, followed by human insulin.

Now that more is known about prokaryote promoters, synthetic DNA can be made, rather than trying to make use of natural promoters in this way.

In eukaryotes, the regulation of gene expression is considerably more complex, and so eukaryote promoters may well not have the intended effect in prokaryotic cells.

What this means in practice, is that if a gene, such as the human insulin gene, is transferred into prokaryote DNA without adding a prokaryotic promoter, it will not be transcribed and hence will not be expressed.

When genes are transferred from eukaryotes to prokaryotes, it is therefore essential that a suitable prokaryote promoter is added to the gene before it forms recombinant DNA with the plasmid vector. The promoter initiates transcription of the gene so that the desired product is expressed.

If eukaryote promoters are to be transferred with eukaryotic genes, into eukaryotic cells of a different species, then care must be taken to ensure that all of the relevant code is included, which may include short base sequences close to the start of the gene (such as TATAAA, [TATA box] within 50 bases of the start of the gene, promotes mRNA formation) or other sequences further away from the gene (such as CACGTG [E box] which binds proteins needed for transcription) some of which may cause the DNA to bend back on itself, so that the promoter is several thousand bases before the gene.

<http://en.wikipedia.org/wiki/Promoter>

Why fluorescent markers (or easily stained substances) are now used instead of antibiotic resistance markers

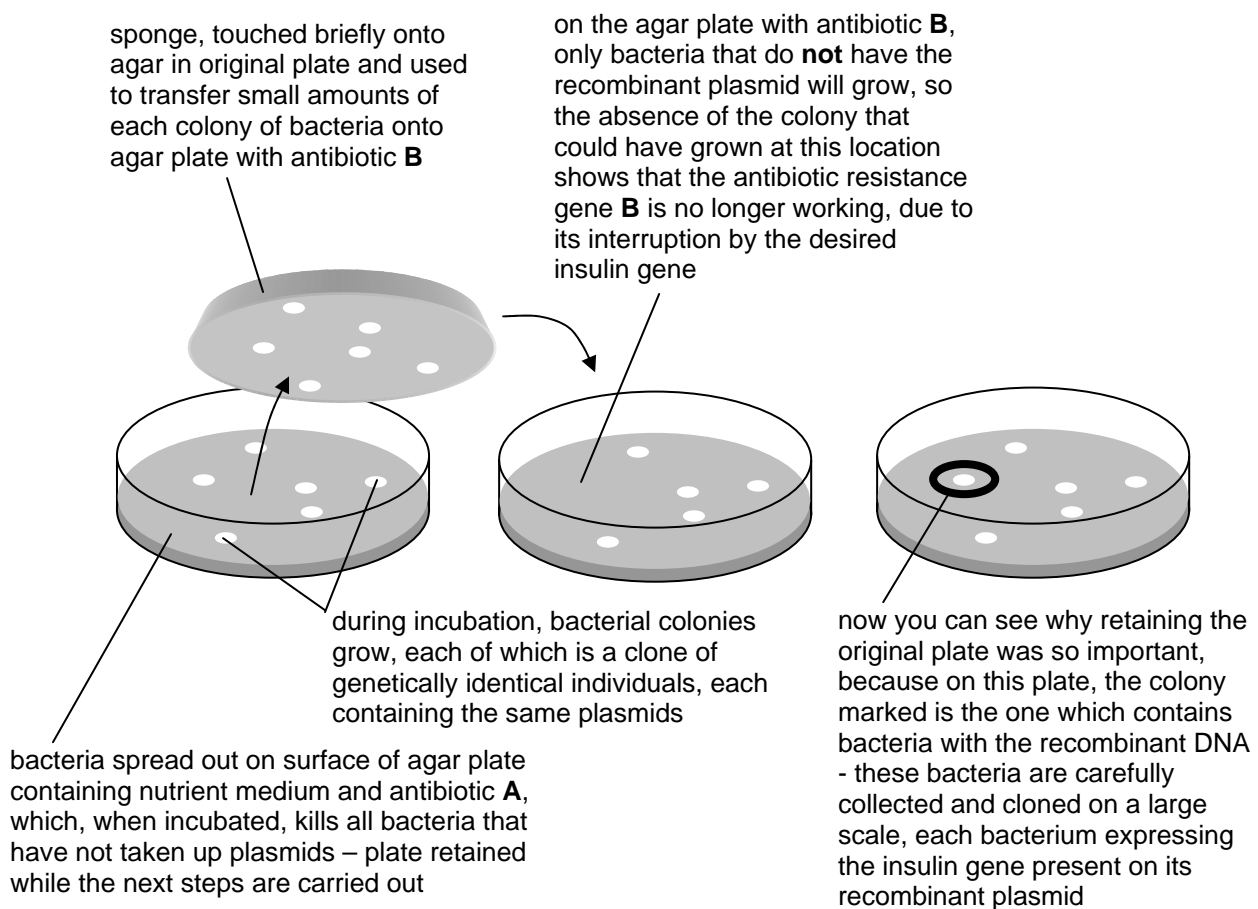
When plasmids containing the human insulin gene are mixed with bacteria, only a small proportion of the bacteria will actually take up the plasmids – this may be as low as 1%. There needs to be some way of identifying those bacteria which have taken up the gene, so that they can be separated from those that have not.

The first methods used were based on antibiotic resistance markers. Not all the bacteria in the culture will successfully take up the plasmid, and not all the plasmids in the mixture will have successfully formed recombinant DNA containing a viable copy of the cDNA insulin gene. The method used to identify the bacteria containing the desired recombinant DNA is:

- The original selected plasmid has antibiotic resistance genes to two different antibiotics, antibiotic **A** and antibiotic **B**. Any bacterium containing this plasmid will grow successfully in the presence of these two antibiotics, but bacteria lacking the plasmid will be killed by the antibiotics.
- The restriction enzyme is selected so that it cuts in the middle of one of these antibiotic resistance genes, in this case the gene for resistance to antibiotic **B**. If a successful recombinant is formed, this one antibiotic resistance gene will no longer work because it is interrupted by the cDNA insulin gene.
- Bacteria that have taken up the plasmid all have a successfully working copy of antibiotic resistance gene **A**. Many plasmids also have a working copy of antibiotic resistance gene **B**, showing that the plasmids have failed to form

recombinant DNA. However, those bacteria that have taken up recombinant plasmids containing the cDNA insulin gene do **not** contain a working copy of the antibiotic resistance gene **B** – which gives a way to identify them as follows:

- The bacteria are spread out and cultured on an agar plate containing antibiotic **A**. Only bacteria that have taken up the plasmid survive and reproduce to form colonies, each of which is a clone, genetically identical to the original cell.
- A sponge is then touched briefly onto the agar, picking up some of the bacteria from each colony. The original agar plate with the colonies is carefully refrigerated to preserve it. The sponge is then touched briefly onto a sterile agar plate containing antibiotic **B**. Bacteria containing recombinant DNA will be killed by this antibiotic, so that their location on the original plate is now known.



One potential problem with using antibiotic markers in this way is that they are present on plasmids, which are commonly transferred between bacteria of the same species and also of different species. This means that if the genetically engineered bacteria come into contact with pathogenic bacteria (e.g. pathogenic strains of *E. coli* or even pathogens that cause TB or cholera) the plasmid, with its antibiotic resistance genes, could be transferred into the pathogen, giving it instant resistance to the antibiotics involved. If this did happen, it would then become much more difficult to control the spread of such bacteria by using these antibiotics. There is no

evidence that such a transfer has ever happened so the risk is a hypothetical one. This contrasts with the known damage caused by routine misuse of antibiotics which selects for naturally resistant bacteria very strongly.

The potential risks led to development of alternative methods of detecting successful genetic engineering. One method, used for example in genetic manipulation of papaya, was to incorporate a marker gene for a protein that fluoresces green under ultra-violet light, along with the desired genes. The genes were added, as is now common in plants, using a micro-projectile to shoot them into the plant cell nuclei. Compared to antibiotic resistance markers, the process has been found to be both quicker and to produce a higher proportion of transformed plants. A commonly used fluorescent protein gene comes from jellyfish.

Another approach is to incorporate alongside the desired gene, another marker gene that produces a harmless product that is easily stained and is not normally produced by the cells. An example of this is the gene for β -glucuronidase (GUS) which produces a harmless product that is easily stained blue. This can be made even safer by linking it to a promoter incorporating a regulator requiring the presence of an unusual material to turn on the gene, which is thus only expressed in the peculiar circumstances of the test. The DNA for the gene and the chemicals required to detect the easily stained product are now widely available and have been used, for example, in detecting successful transformation of fungi.

<http://138.23.152.128/transformation.html> has illustrations of fungi stained with GUS as well as fluorescent markers.

The benefits and hazards of gene technology

Benefits

Through gene technology, it is now possible to produce:

- genetically modified organisms for a specific purpose. Previously, such genetic change would have to be brought about by selective breeding which requires organisms to be of the same species (able to breed successfully together), takes many generations and involves transfer of whole genomes, complete with undesirable background genes. Gene technology is much faster and involves transferring one or few genes, which may come from completely unrelated organisms, even from different kingdoms.
- specific products, such as human insulin and human growth hormone, thereby reducing the dependence on products from other, less reliable sources, such as pig or cow insulin.
- reduce use of agrochemicals such as herbicides and pesticides since crops can be made resistant to particular herbicides, or can be made to contain toxins that kill insects
- clean up specific pollutants and waste materials – bioremediation
- potential for use of gene technology to treat genetic diseases such as cystic fibrosis (see below) and SCID (Severe Combined Immune Deficiency) as well as in cancer treatment.

Hazards

Genes inserted into bacteria could be transferred into other bacterial species, potentially including antibiotic resistance genes and those for other materials, which could result in antibiotic resistance in pathogens, or in bacteria that can produce toxic materials or break down useful materials. Regulation is designed to minimise the risks of escape of such genes. There is little evidence that such genes have escaped into wild bacterial populations.

Crop plants have, by their nature, to be released into the environment to grow, and many millions of hectares of genetically engineered crops, both experimental and commercial, are planted across the globe. So far, fears that they might turn out to be 'super-weeds', resistant to herbicides and spreading uncontrollably, or that their genes might transfer into other closely related wild species, forming a different kind of 'super-weed', or that they might reduce biodiversity by genetic contamination of wild relatives seem to have proved unfounded. A paper was published in *Nature* in 2001 showing that Mexican wild maize populations were contaminated with genes from genetically manipulated maize, but the methods used were flawed and subsequent studies have not confirmed this contamination, suggesting that the wild maize is not genetically contaminated. There is some evidence that Bt toxin, genetically engineered into plants such as cotton and maize, whilst very effective in killing the target species, may kill other, desirable, insects such as bees and butterflies, and may also cause natural selection of Bt toxin resistant insects. Future events may show that such environmental risks are greater than they look at present.

Food that is derived from genetically engineered organisms may prove to be unexpectedly toxic or to trigger allergic reactions when consumed. There is little reliable evidence that this has been so, but the risk remains. Food containing the expressed products of antibiotic resistance marker genes could be consumed at the same time as treatment with the antibiotic was occurring, which would potentially reduce the effectiveness of the treatment. No examples of this are known.

<http://www.ifgene.org/beginner.htm> has a useful summary table of risks and benefits at the end

The social and ethical implications of gene technology

The social impact of gene technology is to do with its potential and actual impact of human society and individuals. In terms of social impact, gene technology could:

- enhance crop yields and permit crops to grow outside their usual location or season so that people have more food
- enhance the nutritional content of crops so that people are better fed
- permit better targeted clean-up of wastes and pollutants
- lead to production of more effective and cheaper medicines and treatments through genetic manipulation of microorganisms and agricultural organisms to make medicines and genetic manipulation of human cells and individuals (gene therapy)
- produce super-weeds or otherwise interfere with ecosystems in unexpected ways, reducing crop yields so that people have less food

- increase costs of seed and prevent seed from being retained for sowing next year (by inclusion of genes to kill any seed produced this way) reducing food production
- reduce crop biodiversity by out-competing natural crops so that people are less well fed
- damage useful materials such as oil or plastic in unexpected ways
- cause antibiotics to become less useful and cause allergic reactions or disease in other unexpected ways

The ethical impact is about the application of moral frameworks concerning the principles of conduct governing individuals and groups, including what might be thought to be right or wrong, good or bad. So in the context of gene technology, it is to do with issues of whether is right or wrong to conduct research and develop technologies, whether it is good or bad. Judgements may be that

- It is good to conduct such research to develop technologies that might improve nutrition, the environment or health
- It is good to use the results of such research to produce food, to enhance the environment or improve health
- It is wrong to continue such research when the potential impact of the technology is unknown and many aspects of it remain to be understood.
- It is wrong to use the results of such research even when the organisms are kept in carefully regulated environments such as sterile fermenters as the risks of the organisms or the genes they contain escaping are too great and unknown
- It is wrong to use the results of such research when this involves release of gene technology into the environment as once it is released it cannot be taken back – the genes are self-perpetuating, and the risks that they might cause in future are unknown

The social and ethical implications of gene technology are complex and relatively unfamiliar to people who are not scientists, including those involved in the media and in government. This complexity and unfamiliarity is the cause of considerable concern and debate. In considering the implications of gene technology the best approach is to avoid the general (e.g. avoid 'it is bad to play God') and stick to the specific and balanced (e.g. it is possible to increase food crop yields with gene technology so more people can be fed, but there is enough food already if it is properly distributed, so people should not be forced to eat products with unknown risks).

To put it in context, In 1976 George Wald, Nobel Prize winning biologist and Harvard professor, wrote: 'Recombinant DNA technology faces our society with problems unprecedented not only in the history of science, but of life on the Earth. It places in human hands the capacity to redesign living organisms, the products of some three billion years of evolution.... It presents probably the largest ethical problem that science has ever had to face. Our morality up to now has been to go ahead without restriction to learn all that we can about nature. Restructuring nature was not part of the bargain.... For going ahead in this direction may be not only unwise but dangerous. Potentially, it could breed new animal and plant diseases, new sources of cancer, novel epidemics.'

Professor David Suzuki who has worked in genetics since 1961 smiles when he reflects on how the certainties which he held in the 1960s have all vanished. He writes "today when I tell students the hottest ideas we had in 1961 about chromosome structure and genetic regulations, they gasp and laugh in disbelief. In 1997, most of the best ideas of 1961 can be seen for what they are - wrong, irrelevant or unimportant..... So what is our hurry in biotechnology to patent ideas and rush products to market when the chances are overwhelmingly that their theoretical rationale will be wrong?"

http://en.wikipedia.org/wiki/Genetic_engineering has a good review of the ethical implications of gene technology
<http://www.biotechnology.gov.au/index.cfm?event=object.showSitemap> click on 'benefits and risks' and then 'arguments for and against gene technology' where you will find an excellent pros and cons review, and a pdf
http://www.bbc.co.uk/religion/ethics/issues/genetic_engineering/index.shtml looks at the ethics of gene technology in the context of the genetic condition, Tay Sachs.
<http://soc.enotes.com/ethics-genetic-article> <http://www.i-sis.org.uk/GE-ethics.php> may also prove useful

The use of electrophoresis in genetic fingerprinting and DNA sequencing

Electrophoresis

Electrophoresis is a method of separating substances and analyzing molecular structure based on the rate of movement of each component in a liquid medium while under the influence of an electric field. In genetic fingerprinting and DNA sequencing, the components being separated are fragments of DNA.

In this case, the type of electrophoresis used is *gel electrophoresis* – the gel appears solid but is actually a colloid in which there are spaces between the molecules through which other molecules can move. Electrodes are placed at either end of the gel, as a result of which the DNA molecules move under the influence of an electric current. Usually the DNA is fragmented (cut across) into a series of fragments using a restriction enzyme or mixture of restriction enzymes. These enzymes cut the DNA at specific restriction sites (see above), but these sites are randomly distributed along the length of the DNA so the fragments are of varied lengths.

The direction of movement depends on the fact that DNA molecules and fragments of DNA are negatively charged and thus move towards the positive electrode (anode). The distance moved in a given time will depend on the mass of the molecule of fragment. The smaller fragments move further in a given time, and the larger fragments of DNA move less far.

Taking humans as an example, almost everyone has 46 chromosomes: 23 pairs if you are female and 22 pairs plus two odd ones if you are male. The longest of these kinds of chromosomes has been numbered as chromosome 1 and the smallest as 22, the sex chromosomes being out of sequence and called X and Y. The base sequence of every chromosome 1 in every human being is similar, but not identical due to the existence of mutations and therefore of different alleles of genes. What this means is that when the DNA is fragmented with a restriction enzyme, the fragments are similar but not exactly the same in DNA from different people.

The DNA is transparent and invisible, so the fragments must be treated to make them visible. There are two key ways of doing this:

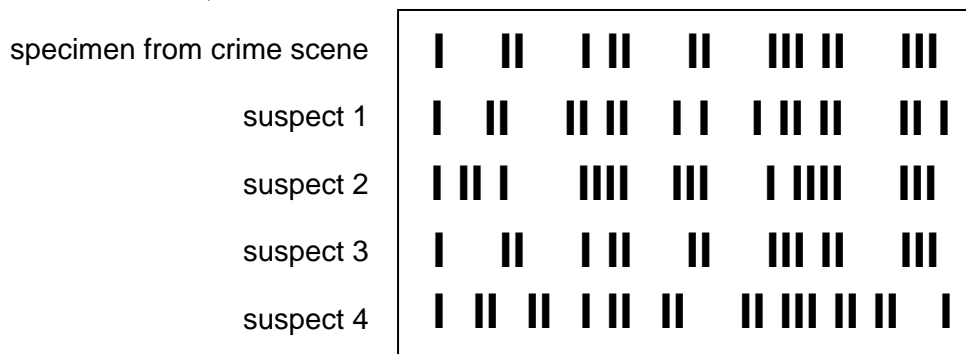
- One is based on staining all of the DNA fragments, for example using ethidium bromide (toxic, fluoresces in short wave UV radiation), methylene blue (fades quickly and stains gel as well as DNA) and Nile blue A (does not stain gel and is visible in ordinary light).
- The other is based on creating a gene probe that is complementary:
 - either to a commonly repeated bit of DNA that will therefore be present on many of the fragments,
 - or to a base sequence that is specific to a particular gene or allele of a gene which will therefore be present on no more than one of the fragments.

The gene probe is a single stranded piece of DNA with a base sequence complementary to the DNA that you wish to identify. In order to make it possible to locate which fragment or fragments the gene probe has attached itself to, the gene probe must be labelled. The most common forms of labelling are:

- to make the probe radioactive and to detect it by its ability to expose the photographic film used to make X-ray photographs
- to stain the probe with a fluorescent stain such as vital red, that will fluoresce with bright visible light when placed in ultraviolet light, making the location of the probe and therefore of the fragment or fragments visible.

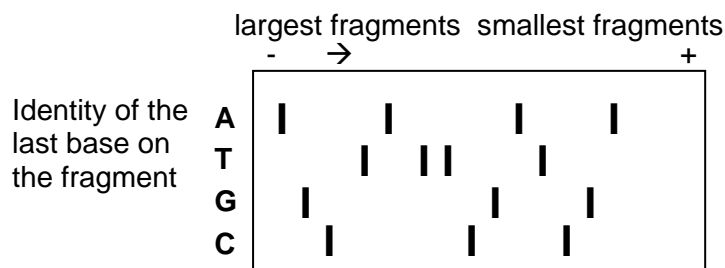
Genetic fingerprinting

Once the DNA fragments have been separated by gel electrophoresis they can be compared with other samples of DNA, thereby allowing determination of the source of the DNA (as in forensic investigations) or whether the samples are derived from related individuals, as shown below:



DNA sequencing

The most publicised example of DNA sequencing is the Human Genome Project. Electrophoresis is used to separate fragments of DNA to enable determination of the order of bases within genes and chromosomes. The fragments vary in length by one base at a time and the last base on each can be identified. Because the fragments are different lengths, they can be separated by electrophoresis as shown below:



base sequence identified by electrophoresis **24** G C T A T T C G A T C G A

The syllabus does not require more detailed understanding of the Sanger method than that electrophoresis is used to separate the fragments of DNA, permitting identification of the bases.

http://www.ornl.gov/sci/techresources/Human_Genome/faq/seqfacts.shtml#how and <http://www.ipn.uni-kiel.de/eibe/UNIT14EN.PDF> have good summaries of the human genome project methods

The causes and symptoms of Cystic Fibrosis

Cystic Fibrosis (CF) is a genetic condition in humans. It is inherited and although it reduces considerably the life expectancy of people with the condition, improved treatments have been helping such people to live longer so that the average life-span is now about 35 years. There are estimated to be around 50,000 people with CF worldwide.

Causes

Cystic fibrosis is caused by several different alleles of a key gene coding for a transmembrane protein that transports chloride ions through cell surface membranes (cystic fibrosis transmembrane regulator, CFTR). Its inheritance is *autosomal* (i.e. it is NOT sex-linked) and *recessive*. The gene is located on chromosome 7. CF alleles originate by mutation of the CFTR protein, but can then be inherited through many generations.

As CF alleles are recessive, individuals with a single copy of such an allele are heterozygous and do not have the condition. There are about 10 million such *carriers* worldwide.

To have CF, it is necessary to be homozygous for CF alleles, most often by inheriting one CF allele from each parent.

Effects of CF

Reduced chloride transport through cell membranes leads to production of thick, sticky mucus that particularly affects the lungs, pancreas and reproductive organs.

- The mucus remains in the lungs rather than being swept out by the tracheal cilia, leading to wheezing and repeated infections. The mucus may be removed by physiotherapy
- The mucus may block the pancreatic duct, preventing amylase and protease enzymes from reaching the small intestine, compromising digestion and nutrition, and also causing a build-up of protease in the pancreas, damaging the pancreatic tissue including the cells that produce insulin, increasing the chance of diabetes.
- The mucus may block the sperm ducts, causing male infertility and may slow the progress of eggs and sperm through the oviducts, reducing female fertility.

<http://www.nlm.nih.gov/medlineplus/ency/article/000107.htm> http://en.wikipedia.org/wiki/Cystic_fibrosis

Progress towards treating Cystic Fibrosis with gene technology

Current treatments for CF deal with the symptoms rather than the causes, for example physiotherapy to remove mucus from lungs, antibiotics to combat recurrent lung infections and enzyme supplements to enhance digestion. These have been very successful in improving people's quality of life and lifespan, but research continues to try and develop techniques for adding functional copies of the CFTR gene to the cells of people with CF.

Since it is a recessive condition, such gene therapy does not need to remove or replace the existing genes in the person's cells – adding a working copy of the gene to a cell and having it expressed would be sufficient to permit that cell to transport chloride ions normally. Since it is the mucus in the lungs that generally limits life-span in people with CF, it is these cells that have been the focus of effort. It is thought that if even a proportion of lung cells could be given a working copy of the gene, this would thin the mucus sufficiently to allow the cilia to operate normally.

The approach that has been trialled with another recessive genetic condition, SCID, is to remove cells from the body, add working copies of the gene and put the cells back. The working copies of the gene integrate themselves into random positions in the genome of the treated cells. The blood cells involved in this case only live for a few weeks so it has to be frequently repeated. Of 14 boys in one French trial, 3 have developed cancer, probably because the gene has been inserted into a critical portion of one of the cells at some point. Clearly this approach cannot be used with CF because the lung surface cells cannot be extracted from the body.

For CF, a vector must be used to deliver the DNA containing the functional CFTR gene into the lung cells.

- *Viral delivery systems* – some viruses such as Adenoviruses can be used as the vector. Normally, viruses which infect lung cells are used – their virulence (ability to cause disease) is removed and they are genetically engineered to carry the functional human CFTR gene.

Early trials have involved either injection with the genetically engineered viruses or inhale them from an aerosol directly into the lungs. The intention is that the lung surface cells are infected with the virus, which releases the genetic material into the cells where it is expressed.

- *Non-viral delivery systems* – other systems are also being developed and have been trialled for safety but have not been used therapeutically e.g.
 1. Creation of a lipid sphere or liposome, containing the DNA. An aerosol is sprayed into the lungs where the liposome will be able to pass through the target cell membrane and carry the DNA into the cell.
 2. DNA can be compressed into a very small volume which may directly enter cells.

Whether the DNA is introduced into the cells by viruses or some other system, the intention is that the gene will be incorporated into the cell's genome and will start to be expressed, to produce CFTR protein to carry chloride ions through its membrane. There is not yet a successful example of treatment of CF by gene therapy. This is because:

- current viral vectors have been found to stimulate allergic or other immune responses
- current liposome vectors have proved inefficient at delivering genes into cells
- the effect of the therapy on chloride ion transport has, so far, lasted only a few days

Research continues to solve these problems to develop a workable treatment for lung symptoms. Further into the future, similar approaches may be possible for pancreatic symptoms. A cure would require every one of the 50×10^{13} cells in the body to be altered, which is not currently thought to be technically possible and would raise significant further ethical issues. To enable people with CF to have children would require germ-line gene therapy where changes are made to human gamete cells that are inherited by the next generation. This would also raise very significant further ethical issues and does not appear to be realistic at present.

http://www.cff.org/about_cf/gene_therapy_and_cf/ <http://www.cfgenetherapy.org.uk/genetherapy.htm>

Genetic screening and counselling

There are now many conditions known to be caused by varied alleles of varied genes and which can therefore be inherited. The pattern of inheritance varies, according to whether the allele is dominant, recessive or sex-linked.

Individuals may be tested for the presence of such alleles – such tests may be requested because there is a history of a particular condition in the family of that person or because the person belongs to an ethnic group which has a high percentage of individuals with a particular allele, such as the alleles that cause Tay Sachs in people who are Ashkenazi Jews.

Genetic screening: The testing of samples of DNA from a group of people to identify the presence or absence of particular alleles and thus the risk of having or passing on particular genetic conditions. Such screening may be:

- *Carrier screening*
 - all the individuals in a family may be screened if one family member develops a particular condition that may be genetic.
 - potential parents may be screened where there is the possibility that one or both of them might carry a recessive allele for some particular condition e.g. cystic fibrosis
- *Prenatal screening* – this is used to determine aspects of the genetic makeup of an unborn child. Such testing can detect a number of genetic conditions:
 - *Chromosomal abnormalities*, such as Down's Syndrome (of particular importance if the mother is over 34), trisomy 13 and trisomy 18.

- *Single gene disorders*, such as haemophilia, sickle cell anaemia and cystic fibrosis
- *Neural tube defects*, such as spina bifida and anencephaly

Pre-natal screening may be carried out in different ways and at different stages of the pregnancy :

- *Chorionic villus sampling* – where the early placental tissue is sampled, usually done at 10 – 12 weeks of the pregnancy
 - *Amniocentesis* – where fetal cells in amniotic fluid are sampled, usually done at 13 – 18 weeks of the pregnancy
 - *Intra-uterine blood test* – where fetal blood is sampled, usually done at 16 – 18 weeks of the pregnancy
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- *Newborn screening* – in some countries, all newborn babies are screened for genetic conditions such as phenylketonuria (pku) by a simple blood test. This test enables the affected individual to be put onto a protective diet low in the amino acid phenylalanine, for the rest of their life, to protect them from the damaging symptoms of the condition.

http://en.wikipedia.org/wiki/Genetic_testing

Once the results of a genetic test are known, it will be necessary for those involved to receive *Genetic Counselling*. This will involve an explanation of the results and the implications in terms of probabilities, dangers, diagnosis, and treatment.

- For the individual – depending on the nature of any detected allele (dominant or recessive), it will be necessary to explain the possible future consequences in terms of the health of the individual and whether this is likely to have repercussions on their education or employment. In some cases, it might affect their prospects of obtaining insurance.
- For couples who want to have children – again, depending on the nature of the inheritance, it will need to be explained what the probabilities are of any children inheriting the defective allele – and the chances of any child actually having the disease i.e. it showing in their phenotype. All of this will depend on whether the allele is dominant, recessive or sex-linked.

In addition to the practical considerations of genetic screening and counselling, there are also some ethical considerations :

- Who decides who should be screened or tested?
- Which specific disorders should be screened?
- Who should be providing the screening?
- Should we screen or test for disorders for which there is no known treatment or cure?
- What psychological impact might the results have on the individuals involved?
- Should the results be confidential?
- If not, who should be able to have access to the information?
- Should the results be made available to potential employers, insurers etc.?

http://en.wikipedia.org/wiki/Genetic_counseling

http://www.jmu.edu/vmic/McKown_pharm_article.pdf is useful overview of biotechnology

<http://www.ncbe.reading.ac.uk/NCBE/MATERIALS/menu.html> is a great source of materials for doing practical work in this area

Gene Technology Self-Assessment Questions

SAQ 1 Outline the steps involved in the transfer of the human insulin gene into E.coli bacteria.

SAQ 2 Explain what is meant by a fluorescent marker and why they are considered to be preferable to markers which confer antibiotic resistance.

SAQ 3 List three advantages of treating diabetics with genetically engineered human insulin, rather than pig or cow insulin.

SAQ 4 Describe the use of electrophoresis in genetic fingerprinting.

SAQ 5 With reference to Cystic Fibrosis,

(a) Explain its pattern of inheritance

(b) Outline the use of gene technology in the possible treatment of Cystic Fibrosis.