A Level Science Applications
Support Booklet: **Biology**

UNIVERSITY of CAMBRIDGE
International Examinations

Applications of Biology

Contents List

INTRODUCTION

Too often the study of Biology at A-level can seem like a disorganised collection of facts. The problem is that to understand many of the ways in which Biological knowledge, understanding, skill and technology is used to enhance our lives, we need to understand the basic principles of Biology. This is one of the purposes of the core sections of the biology syllabus, and takes time and effort. Students who are approaching the end of their A level studies have acquired a better understanding of the way Biology works and can thus look at applications of Biology in a more meaningful way.

This booklet is designed to support teaching and learning in 9700 Biology A Level, in the Applications of Biology section of the syllabus which is a feature of the 2007 syllabus and those that follow. This material replaces the former options. All students are expected to study all sections of the Applications of Biology syllabus.

This section of the syllabus examines some of the important areas in which facts, understanding, skills and technology obtained from biological science research and studied in the core syllabus can be applied to making life better. The topics include biodiversity and conservation, gene technology, biotechnology, crops plants and aspects of human reproduction.

Chapter 1: Biodiversity and Conservation investigates the variety and classification of organisms and the potential and actual advantages of such biodiversity. Through the study of the African elephant as an example, the reasons that organisms become endangered are considered. The role of zoos, botanic gardens, national parks and seed banks are considered in relation to conservation of biodiversity.

Chapter 2: Gene Technology takes a structured look at the process known as genetic engineering or genetic manipulation and the creation of recombinant DNA and its implications. Electrophoresis is used as a context to consider genetic fingerprinting and DNA sequencing. Cystic fibrosis is investigated as a genetic condition which gene technology has the potential to improve.

Chapter 3: Biotechnology is about the variety of industrial applications of microorganisms and cultured cells. It includes bio-extraction from heavy-metal ores, culture of microorganisms focussing on penicillin, enzyme immobilisation, application of such enzymes in dip-sticks and biosensors for glucose detection and monoclonal antibodies.

Chapter 4: Crop Plants takes a look at the variety of techniques that are used to enhance the crop plants that feed the world. Aspects of the biology and reproduction of crop plants are considered as well as some of their adaptations and use of hybridisation and genetic manipulation to improve them.

Chapter 5: Aspects of Human Reproduction reviews human gametogenesis and the role of hormones in controlling the menstrual cycle. The role of hormones and synthetic hormones in controlling fertility is exemplified by the combined contraceptive pill and IVF, looking both at how they work and also their implications.

Each topic lays out the key objectives, stating concisely the intended learning outcomes of the chapter, which are detailed in the syllabus, to which reference should also be made. It has key definitions that include any hard-to-define terminology in the syllabus section. These are followed by the main text, termed Key Ideas. At the end of each chapter are self-assessment questions to help students to check their understanding as they work through the material.

The book is designed so that it can be used by teachers, alongside the Applications of Biology syllabus and the Scheme of Work that can be found on the CIE Teacher Support Website (contact international@cie.org.uk to find out how to gain access to this learning resource). The book should help teachers to design effective learning programmes to teach this material, which makes up 16% of the total assessment at A level and should thus make up just over 30% of the total teaching time available during the A2 part of the course.

The book is also designed so that it can be used by students, to promote their own learning, and for this purpose contains, as well as a lot of detailed and carefully chosen information, self-assessment questions for students to use in helping to determine how effective has been their learning.

Throughout the book are given website addresses. All of these have been tested and are working at the time of writing, but such sites are notoriously quick to change their url addresses, so by the time that you get to try and use them, they may well be found not to work. The author strongly recommends that a good search engine such as google or yahoo, or a meta-search engine such as copernic, be used to seek up-to-date websites for information on each of the topics on the syllabus. Make use of the ability of these sites to search for definitions of terms (e.g. search for ‘biodiversity definition’), specific information (e.g. searching for ‘five kingdom classification’ in google produces over 10 million hits, of which every site on the first page is relevant and useful) and images (e.g. search for ‘maize leaf’ in the image tab on google currently gives 446 hits, on the first page of which are eight useful images).

BIODIVERSITY AND CONSERVATION

Key Objectives

- To be able to outline the five kingdom classification of living organisms
- To be able to define the term *biodiversity* and discuss its meaning
- To understand why *biodiversity* is declining and the need fore it to be maintained
- To understand the reasons why one named species has become endangered
- To understand the ways in which endangered species may be protected and prevented from becoming extinct

Key Definitions

- *Biodiversity* – this is the total number of different species living in a defined area, ecosystem or biome. It is also possible to consider the biodiversity of the earth.
- *Endangered species* – any species whose numbers have become so low that they are unlikely to be maintained by normal rates of reproduction and are in danger of becoming extinct.

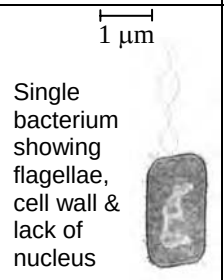
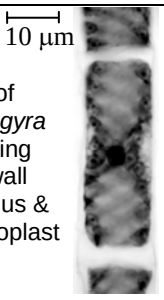
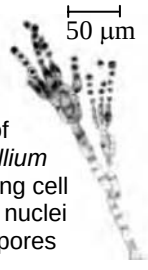
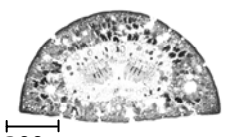
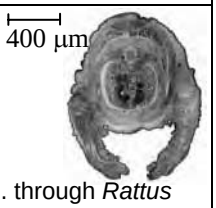
Key Ideas

The Five Kingdom Classification

To understand the five kingdom classification, pupils will need to be familiar with Syllabus section A, especially c, e and g – the structure of plant and animal cells and the differences between prokaryotes and eukaryotes.

Early classifications suggested that organisms could best be organised into two kingdoms, those having no cell walls, whilst being motile and heterotrophic (animals) and the rest (plants). It was realised that this did not work very well as some of the organisms in the 'plant' kingdom were very different, for example prokaryotic bacteria and highly differentiated eukaryotic flowering plants. Various alternative classifications have been proposed, most recently a three-domain classification in which prokaryotes are divided into two domains (bacteria and archaea) with eukaryotes as a third. http://en.wikipedia.org/wiki/Three-domain_system The working classification used by many biologists, and featured in the 9700 biology syllabus has five kingdoms and is considered below. http://en.wikipedia.org/wiki/Five-kingdom_system

This five kingdom classification divides the living world into five Kingdoms. The diagnostic features of each of these kingdoms is summarised in the table on the following page.

Prokaryotae (prokaryotes)	**Protoctista** (protoctists)	**Fungi**	**Plantae** (plants)	**Animalia** (animals)
single-celled or groups of cells cells are prokaryotic i.e. • have no nucleus or membrane-bound organelles, • circular DNA with no associated histone proteins • small circles of DNA (plasmids) often present • small (70s) ribosomes	single-celled or not differentiated into tissues – any eukaryote not in one of the other three eukaryote kingdoms cells are eukaryotic i.e. • have a nucleus and various membrane-bound organelles, • DNA in form of linear chromosomes with histone proteins in a nucleus • larger (80s) ribosomes diverse, some have cell walls, some don't, some autotrophic, some heterotrophic	most are multicellular but not differentiated into tissues, yeasts are single-celled never motile (self-propelled) cells are eukaryotic i.e. • have a nucleus and various membrane-bound organelles, • DNA in form of linear chromosomes with histone proteins in a nucleus • larger (80s) ribosomes cell walls of chitin heterotrophic body made of hyphae, reproduce by spores	multicellular and differentiated into tissues motile male gametes in mosses and ferns cells are eukaryotic i.e. • have a nucleus and various membrane-bound organelles, • DNA in form of linear chromosomes with histone proteins in a nucleus • larger (80s) ribosomes cell walls of cellulose autotrophic (feed by photo-synthesis)	multicellular and differentiated into tissues often motile cells are eukaryotic i.e. • have a nucleus and various membrane-bound organelles, • DNA in form of linear chromosomes with histone proteins in a nucleus • larger (80s) ribosomes no cell walls heterotrophic
bacteria archeobacteria cyanobacteria	algae protozoa slime moulds	yeasts moulds mushrooms	mosses ferns conifers flowering plants	worms arthropods chordates
 Single bacterium showing flagellae, cell wall & lack of nucleus	 Part of *Spirogyra* showing cell wall nucleus & chloroplast	 Part of *Pencillium* showing cell walls, nuclei and spores	t.s. through *Pinus* leaf showing various differentiated tissues 	 t.s. through *Rattus* thorax showing various differentiated tissues

Biodiversity

The biodiversity of the planet is the result of evolution. In any ecosystem, there is a huge interdependence between species and it is clear that biodiversity is essential to maintain ecological balance and stability.

Another part of biodiversity is the extent of genetic diversity with species and populations. Such genetic diversity is also essential for the stability and survival of a species.

The Need to maintain Biodiversity

Biodiversity is in decline – mostly as a result of a variety of man's activities. It is now well understood that it is important to try and halt this decline – indeed, conservation measures are needed, not only to halt the decline, but to try and restore as much biodiversity as possible.

The need to maintain biodiversity may be considered in terms of biological reasons or reasons from a human perspective :

Biological reasons

As mentioned above, it is essential that biodiversity is maintained if ecosystems (and the whole planet) are to remain ecologically balanced and stable.

In addition, evolution has resulted in diverse gene pools within populations – the maintenance of these gene pools and the genetic diversity of species is extremely important if species are to be prevented from becoming extinct.

Human reasons

Other species of animals and plants provide an important resource for humans. These may be

- For use in agriculture, either as potential food supplies or to be crossed with existing agricultural species to improve features, such as yield, hardiness or disease resistance.
- To provide possible medicines
- To encourage tourism in some countries - ecotourism
- From an ethical point of view, if human activity has been largely responsible for the decline in biodiversity, then humans have an obligation to reverse this decline. Equally, it is important to try and maintain the current level of biodiversity for future generations.

Reasons why species have become endangered

In African ecology, the elephant is regarded as a keystone species. In 1930 there were estimated to be 5 – 10 million African elephants. By 1979 the number was reduced to 1.3 million and when it was officially added to the endangered list in 1989,

the numbers had fallen to around 600,000 - less than 10% of its numbers earlier in the twentieth century.

http://www.panda.org/news_facts/education/high_school/species/herbivores/african_elephant/index.cfm
http://www.awf.org/wildlives/elephant.php http://www.save-the-elephants.org/

A number of factors contributed to this dramatic decline in numbers:

- *Habitat loss* – elephants eat a great deal and need a large amount of habitat. During the twentieth century, the human population of Africa has increased massively and, as a result, humans and elephants have become competitors for living space. The forest and savannah habitats of the elephant have been reduced as humans have used timber for fuel and building and land for growing crops and grazing livestock.
- When humans and elephants live in close proximity, various problems arise – elephants raid crops and, on occasion, will rampage through villages. Farmers and other residents regard them as something of a pest and shoot them.
- *Hunting* – this has been a major cause of the decline in elephant numbers. Elephants became prized trophies for big-game hunters and, more recently, they have been killed for their ivory tusks. Ivory is easily caved and regarded as a beautiful material – most of the ivory carving in the world takes place in Japan and other countries in Asia. At one stage, ivory was more expensive than gold – indeed, it became known as 'white gold'. Hunting continues for the global 'bushmeat' trade (see http://www.bushmeat.org/IMAP/species/L_africana.htm).
- *Poaching* – it is no longer legal to hunt elephants in most African countries. However, the high prices paid for ivory meant that elephants continued to be killed by poachers. At its peak, the poachers became highly organised, using automatic weapons, vehicles and even planes to herd and kill huge numbers at a time. The biggest elephants were usually targeted (because they have the largest tusks) which meant that it was generally the adults that were killed, leaving young elephants without any adults to learn from. As a result, the social structure of the elephant populations broke down and many of the elephant groups left were leaderless juveniles.

African elephants - summary

- IUCN Red list status = vulnerable
- CITES Listing Appendix I except Botswana, Namibia, South Africa and Zimbabwe, Appendix II
- Habitat loss – competition between humans and elephants for space, trees and grazing leading to loss and fragmentation of the elephant habitat
- Hunting and poaching – for trophies, ivory, protection of villages and for bushmeat

Other species which are endangered include the orangutan
http://www.un.org/works/environment/animalplanet/orangatang.html
http://www.panda.org/about_wwf/what_we_do/species/our_solutions/endangered_species/great_apes/orangutans/index.cfm
http://www.orangutan.org/facts/orangutanfacts.php

Methods of protecting endangered species

There are a variety of ways in which attempts are being made to protect endangered species and prevent them becoming extinct. The extent to which these attempts are successful is somewhat variable.

- **Zoos**

  One way of protecting endangered species of animals is to capture some from the wild and place them in captivity. In this way, it is possible to make sure that they are well fed, protected from predators and disease and isolated from other potential problems which might be encountered in their natural habitat.

  If such animals are simply placed in zoos, the zoo is really acting as an 'ark' and little is actually being achieved in terms of maintaining or increasing populations in the wild. If the animals will breed in captivity, then it is possible to maintain or even increase numbers. If such captive-bred individuals can be returned to their natural habitat, then it might be possible to increase numbers in the wild, thereby preventing the endangered from becoming extinct.

  Captive breeding has a number of advantages:

  - it is possible to monitor the health of the mother and the development of the fetus during the pregnancy.
  - sperm and eggs can be obtained from the captive individuals
  - these can be stored in a frozen form
  - it allows the possibility of artificial insemination
  - also in-vitro fertilisation
  - fertilised embryos may be implanted in surrogate mothers (which might even be of different species)
  - there is the possibility of international co-operation and the transfer of breeding individuals between different zoos
  - it allows the keeping of breeding records and the genetic relatedness of captive individuals

  Golden lion tamarin http://www.hrw.com/science/si-science/biology/animals/glt/index.html
  http://nationalzoo.si.edu/ConservationAndScience/EndangeredSpecies/GLTProgram/
  Arabian oryx http://www.arabian-oryx.com/

  Although some species of animals have been bred successfully in captivity and released back into the wild, with other species this has not been straightforward and a number of problems have been encountered. It has been found that some species simply do not breed successfully in captivity, whilst, in some cases, there have been problems in releasing animals that have bred in captivity.

  Captive Breeding

  There are a number of reasons why animals do not always breed successfully when in captivity:

  1 They are no longer living in their natural habitat

2 The conditions experienced in captivity can cause stress and behavioural changes
3 The stress can disrupt normal reproductive cycles and breeding behaviour
4 They often have little choice of mate and may reject the chosen mate

Release of captive-bred individuals into the wild

Problems which reduce the success rate of releasing captive-bred individuals include :

1 Habitat destruction (usually as a result of man's activities) might mean that there is very little suitable habitat available in which to release the animals
2 Having been in captivity, animals might not find it easy to move around in their natural habitat
3 It may not be easy for them to find enough food – especially if they have been used to being fed in captivity
4 They may not be able to communicate with other members of their species in the wild and may not integrate into social groups
5 They may be susceptible to diseases in the wild

Some of these problems are being overcome by making sure that conditions within zoos are as close to the natural habitat of the species as possible. Contact with humans is kept to an absolute minimum and individuals can be 'acclimatised' in cages before they are actually released into their natural habitat.

Cheetah http://nationalzoo.si.edu/ConservationAndScience/EndangeredSpecies/Cheetah/
Californian condor
http://bna.birds.cornell.edu/BNA/account/California_Condor/CONSERVATION_AND_MANAGEMENT.html
Detailed overall review of Captive Breeding and Reintroduction
http://darwin.bio.uci.edu/~sustain/bio65/lec23/b65lec23.htm

- **Botanic gardens**

Endangered species of plants can be grown in botanic gardens. Clearly, it is possible to create ideal growing conditions – either outdoors or in glasshouses, when it is possible to control very carefully the growing conditions. This applies to the availability of light, nutrients, water and the atmospheric conditions.

Within such botanic gardens, it is also possible to propagate endangered species – either by growing from seed or by some means of vegetative propagation, such as cuttings. Techniques of tissue culture also allow large numbers to be produced very quickly.

This allows the possibility of re-introducing endangered species of plants into their natural habitat.

Botanic Gardens Conservation International http://www.bgci.org.uk/
Varied links to botanic garden websites http://nature.ac.uk/browse/580.73.html
Royal Botanic Gardens, Kew, London, UK http://www.rbgkew.org.uk/conservation/index.html

- **Seed banks**

Many plants produce seeds which are very long-lived and large numbers can be stored in a relatively small space. Such a collection of seeds is referred to as a seed bank. The life span of such seeds can be extended if they are kept in carefully controlled conditions – especially in an atmosphere of low oxygen levels, moisture and temperature.

Given that the seeds will contain all the genetic material of any given species, it also means that the gene pool of that species is being maintained.

Clearly, if the seeds of endangered species are stored in this way, such seeds can be germinated at any time and plants can be grown in Botanic gardens or restored to the wild.

Some species produce seeds which have a limited longevity (e.g. cocoa, rubber, coconut) – keeping their seeds in seed banks is not possible. Such plants would need to be maintained in botanic gardens.

http://www-saps.plantsci.cam.ac.uk/osmos/os24.htm http://www.rbgkew.org.uk/msbp/

- **National Parks (and other protected areas)**

Many countries have designated areas, such as National Parks, which are set up to conserve rare / endangered species and maintain important habitats. Often, legislation is passed to ensure that such areas are protected under the law.

The ways in which National Parks protect their resident species include :

1. Wardens, rangers and volunteers can be used to patrol the parks
2. Access by humans can be restricted – often footpaths are created and maintained to avoid interference with wildlife habitats
3. Agricultural activities can be strictly controlled – traditional farming methods can be encouraged
4. Industrial activities and mining can be limited and controlled
5. The building of roads, dwellings and other developments can be strictly controlled
6. Visitor Centres can be established to educate the general public in the importance of conservation within the Park – and elsewhere
7. Wildlife can be protected directly e.g. 24 hour surveillance of nests / breeding sites

In addition to National Parks (which usually occupy large areas of land), different countries can also create other categories of conservation areas if they contain species or habitats which need some form of protection

http://www.wcmc.org.uk/protected_areas/data/sample/iucn_cat.htm http://www.unep-wcmc.org/protected_areas/index.html

Biodiversity and Conservation Self-Assessment Questions

SAQ 1 With reference to the five kingdom classification, outline the similarities and differences between :

(a) prokaryotes and protoctista

(b) fungi and plants

(c) plants and animals.

SAQ 2 Explain what is meant by the term *biodiversity* and give **three** reasons why it is important that it should be maintained.

SAQ 3 With reference to a **named** endangered species, explain why its numbers have declined to a level at which it had to be placed on the endangered list.

SAQ 4 Outline the roles of zoos and botanic gardens in the protection of endangered species.

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.

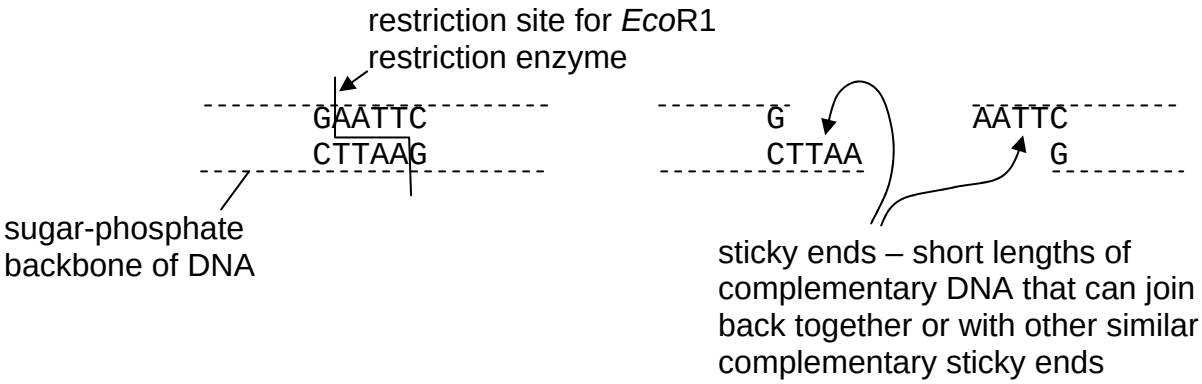
Key Ideas

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

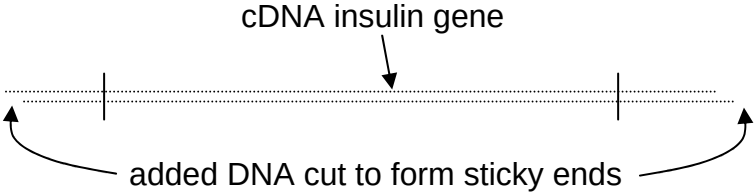
1 Human insulin gene must be identified. There are various ways in which this might have been done, described at the end of this section. What was actually done, in the late 1970's, was as follows:

- 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)

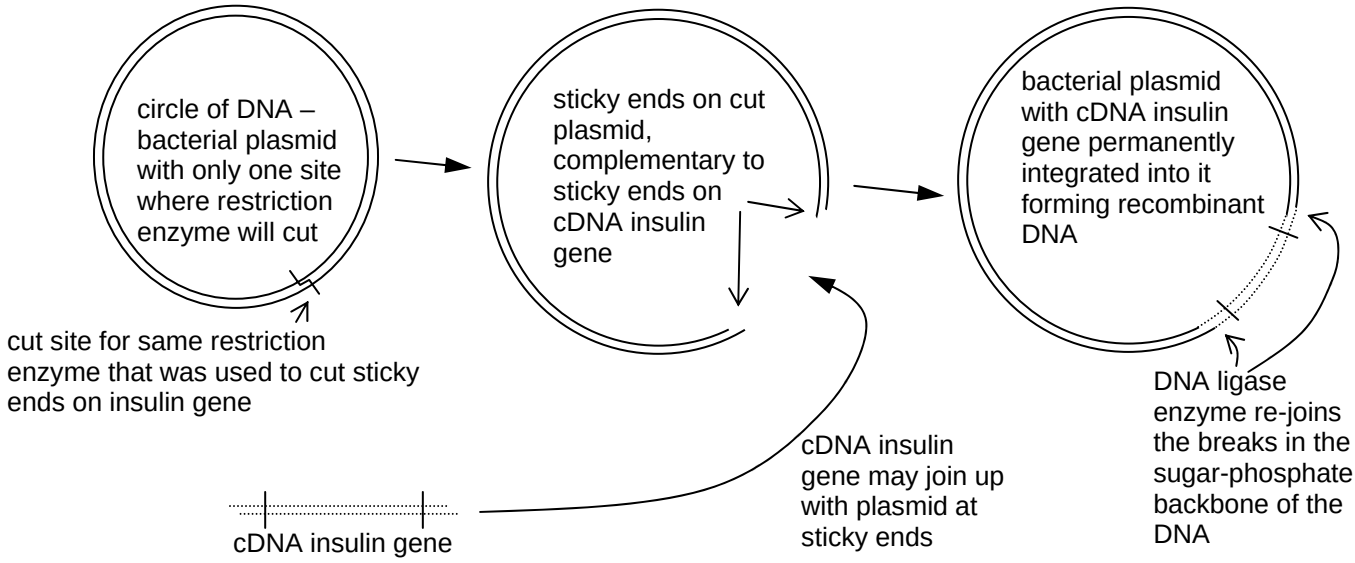
2 Additional, non-coding DNA was added to the ends of the cDNA insulin genes so that 'sticky ends' could be produced using *restriction enzymes* (also called restriction endonucleases). Restriction enzymes cut DNA at specific base-sequences – their restriction site, for example, *Eco*R1 cuts at the sequence GAATTC. Some restrictions 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

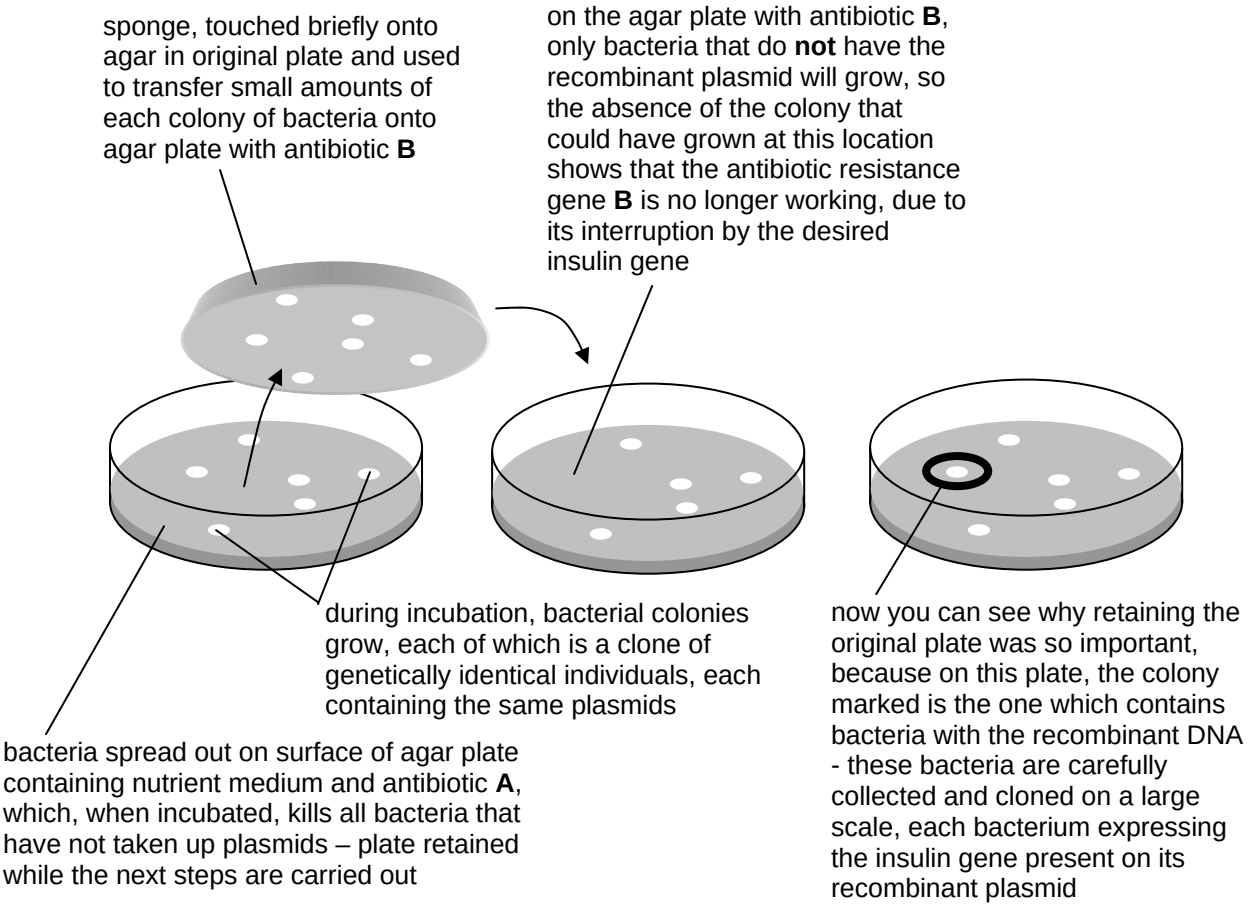
When plasmids containing the human insulin gene are mixed with bacteria, only a small proportion of the bacteria will actually take up the plasmids – this may be as low as 1%. There needs to be some way of identifying those bacteria which have taken up the gene, so that they can be separated from those 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 markergene 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 ß-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, geneticially 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:

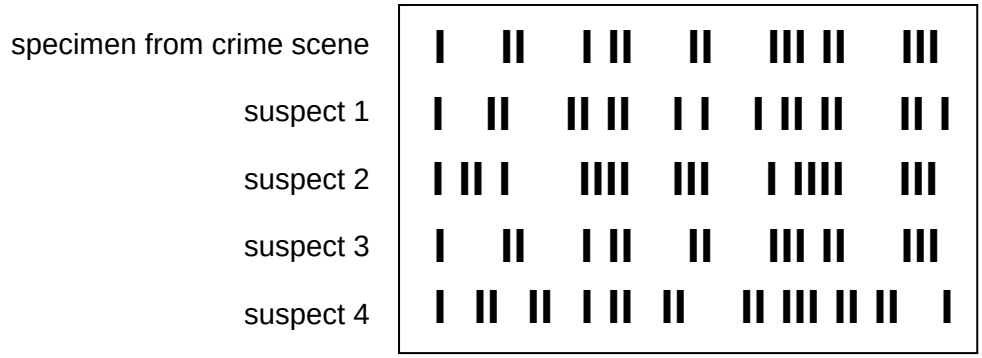
- One is based on staining all of the DNA fragments, for example using ethidium bromide (toxic, fluoresces in short wave UV radiation), methylene blue (fades quickly and stains gel as well as DNA)and nile blue A (does not stain gel and visible in ordinary light).
- The other is based on creating a gene 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.

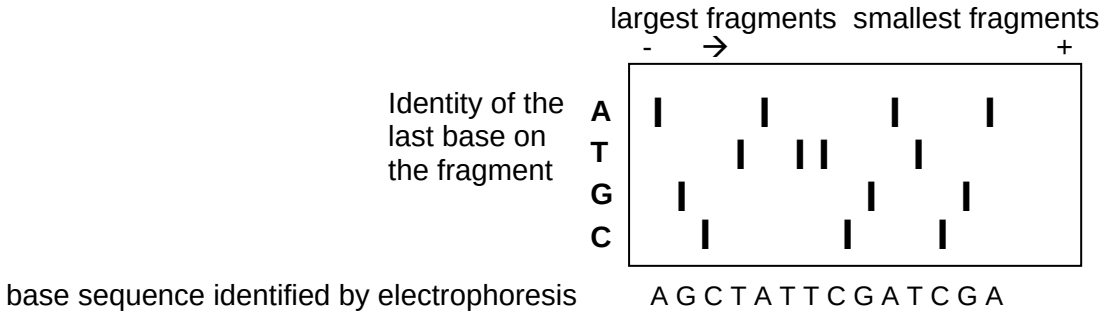
Genetic fingerprinting

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:



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 Downs 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

- *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.

BIOTECHNOLOGY

Key Objectives

- To be able to describe the use of microorganisms in the extraction of heavy metals from low grade ores

- To understand the terms *Batch Culture* and *Continuous Culture* and to be able to compare the advantages and disadvantages of each type of culture with reference to the production of penicillin, protease enzymes and mycoprotein

- To be able to describe for penicillin (as an example of an antibiotic) its mode of action on bacteria and why it has no effect on viruses. Also, the causes and effects of antibiotic resistance.

- To immobilise an enzyme in alginate and compare this with the use of an enzyme that has not been immobilised

- To be able to explain the use of dip sticks and biosensors in the quantitative measurement of glucose

- To be able to outline the hybridoma method in the production of monoclonal antibodies

- To evaluate the use of monoclonal antibodies in the diagnosis and treatment of disease and testing for pregnancy

Key Definitions

- *Batch Culture* – when fermentation is carried out in a closed or batch fermenter. Microorganisms and nutrient medium are added to the fermenter and left for a period of time. During the process, nothing is added to or removed from the fermenter (except for the venting of waste gases). The product is separated from the mixture at the end. Temperature is controlled and nutrients are usually depleted at the end.

- *Continuous Culture* – when fermentation is carried out in an open fermenter. Nutrients are added and product removed at a steady rate throughout the process. This will maintain the microorganisms at the exponential phase of growth. It is very important to monitor pH, temperature and oxygen concentration as well as levels of nutrients and product. All of these should be kept constant.

- *Antibiotic* – a chemical substance, produced by a microorganism (bacterium or fungus) which will inhibit the growth or replication of other microorganisms.

- *Enzyme immobilisation* – the attachment of enzymes to insoluble materials, which then provide support for the enzymes. This allows enzymes to be held

in place throughout the reaction, following which they are easily separated from the products and may be used again.

- *Biosensor* - a device which makes use of a biological molecule (or sometimes a cell) to detect and measure a chemical compound.

- *Monoclonal antibodies* – these are identical antibodies, produced to be effective against a single, specific antigen.

Key Ideas

The use of microorganisms to extract heavy metals from low grade ores

Some bacteria are described as chemoautotrophic i.e. they derive their energy via the breakdown of inorganic chemicals. In some cases, this involves the breakdown of ores of heavy metals, as a result of which the metal itself is released.

Metals which can be extracted in this way include copper, uranium, cobalt, lead, nickel and gold.

Bacteria of the genus Thiobacillus are commonly used in this process e.g. Thiobacillus ferrioxidans is sued in the extraction of copper and uranium.

Some species of bacteria actually accumulate metals. This property can be made use of, both in the extraction of metals and in the detoxifying of waste. For example, some species of Pseudomonas are known to accumulate mercury and uranium, while some species of Thiobacillus accumulate silver.

http://en.wikipedia.org/wiki/Bioleaching http://www.spaceship-earth.org/REM/BRIERLEY.htm

Compare the advantages and disadvantages of batch and continuous cultures in the production of secondary metabolites (such as penicillin), enzymes (such as proteases) and biomass (such as mycoprotein)

In general, batch culture methods have certain advantages over continuous culture. These are:

- the culture is easy to set up
- the environmental conditions are relatively easy to control
- the types of vessels used can be used for different processes at different times
- if the culture becomes contaminated, it is only one batch that is lost
- the level of nutrients drops, which can create the conditions necessary for the microorganism to manufacture secondary metabolite such as penicillin

Some advantages of continuous culture are:

- it can be carried out in smaller vessels, given that the microorganisms are maintained in the exponential phase and productivity is therefore high
- the high productivity for biomass and intra- and extra-cellular enzymes is more cost effective

However, some disadvantages of continuous culture are:

- microbial growth, clumping of cells and foaming can tend to block up inlet pipes
- it can be difficult to control all the environmental factors – if they are not controlled adequately, there can be a considerable amount of waste
- it is not possible to create the low-nutrient, high-stress conditions under which secondary metabolites such as penicillin are produced

http://textbookofbacteriology.net/growth.html
http://www.biologymad.com/master.html?http://www.biologymad.com/Biotechnology/Biotechnology.htm

The mode of action of penicillin on bacteria (and why it does not affect viruses)

In general, antibiotics bring about their effects in a number of ways :

- inhibiting protein synthesis – interfering with transcription or translation.
- interfering with the synthesis of bacterial cell walls – such antibiotics are only effective when the bacteria are growing.
- interfering with the functioning of the cell membrane – the bacteria will lose it's ability to control the uptake or removal of water and other molecules.
- inhibiting enzyme activity – this will disrupt metabolism.

Penicillin is described as a *broad spectrum* antibiotic i.e. it is effective against a wide range of bacteria. It is one of the antibiotics which work by interfering with the synthesis of new cell walls. Specifically, it inhibits the enzymes involved in the synthesis of cross-links between the peptidoglycan polymers in bacterial cell walls.

Some bacteria possess enzymes that break down penicillin (penicillinases) – this makes them resistant to such antibiotics e.g. all strains of Mycobacterium tuberculosis are resistant to penicillin.

Viruses do not have any form of cell structure or metabolism – hence, antibiotics are ineffective against viruses. Viruses replicate only within the living host cells, and make use of the living host cell's transcription and translation mechanisms. These are eukaryotic mechanisms and thus are not affected by any antibiotics, which affect only prokaryotic mechanisms. The absence of any sort of cell wall means that penicillin has no effect on viruses.

http://www.elmhurst.edu/~chm/vchembook/652penicillin.html
http://en.wikipedia.org/wiki/Beta-lactam_antibiotic

The causes and effects of antibiotic resistance

The development of antibiotic resistance in bacteria is an example of the process of natural selection.

Bacteria, like all organisms, are genetically variable. These variations occur by natural mutation giving rise to new alleles of genes. Natural selection can change the frequency of these alleles in the population so that most of the bacteria in the population are resistant, by the following steps:

- Within natural populations of bacteria, some individuals have alleles of genes which give resistance to a particular antibiotic
- bacteria cause an infection, leading to treatment of the infected person with antibiotic
- the antibiotic will kill susceptible bacteria, but resistant bacteria will survive
- only the resistant bacteria will reproduce, resulting in an increase in the frequency of the bacteria that are resistant to that particular antibiotic
- there will be an increase in allele frequency for the allele of the gene that gives resistance in the population of bacteria
- people infected in the future are infected by bacteria more likely to carry the alleles for resistance

During the second half of the twentieth century, there was an increasing and widespread use of a whole range of antibiotics. The result has been that many strains of bacteria have been established which are resistant to a variety of antibiotics.

The alleles of genes that cause resistance arise for the first time by mutation. Such genes are often located on plasmids, which means that they can rapidly spread from one bacterial species to another since plasmids are naturally exchanged between species. The plasmids may also contain a number of different antibiotic resistance genes so that species can suddenly acquire resistance to a number of antibiotics when before they had none. Such populations of bacteria that are now resistant to a number of different antibiotics, may cause infections that are extremely difficult to treat.

This has meant that it is constantly necessary to try and create new types of antibiotics, to which the bacteria are not resistant. However, once these new antibiotics are in use, it is often not long before resistant strains of bacteria are established. It is proving to be almost impossible to keep ahead of the development of antibiotic resistance by bacteria.

http://en.wikipedia.org/wiki/Antibiotic_resistance
http://www.biologymad.com/master.html?http://www.biologymad.com/Evolution/Evolution.htm

The immobilisation of an enzyme in alginate. The ease of recovering the enzyme and purifying the product compared with using an enzyme that has not been immobilised

Enzymes can be immobilised in a variety of ways. One way is to trap the enzyme molecules in alginate beads – amylase is often immobilised in this way. The process of immobilisation involves the following stages:

- amylase enzyme is mixed with a solution of sodium alginate
- this mixture is dripped (usually through a syringe) into a solution of calcium chloride
- the sodium ions are displaced by the calcium ions, resulting in the formation of hard, insoluble beads of calcium alginate, in which are trapped the molecules of amylase

- the alginate beads are left to harden further, following which they are rinsed

Following the formation of the beads in this way, they are normally placed in a suitable container to create a column of beads. A suspension of starch can then be trickled down the column and collected in a beaker. If the contents of the beaker are analysed, it is found to be a solution of maltase, without any starch being present i.e. the starch has been hydrolysed by the amylase as it passed through the beads.

By hydrolysing the starch in this way, there is no contamination of the product with enzyme – and the amylase remains in the beads, which can then be used again. This is the case whenever it is possible to use immobilised enzymes.

If the enzyme is not immobilised in this way, the product will contain enzyme molecules and a further stage will be required to recover them.

advantages of immobilisation:

- enzyme can be recovered after use using a very coarse filter rather than a molecular filter
- enzyme does not contaminate product
- immobilisation may enhance stability (thermostability or pH-stability) of the enzyme molecule as it is supported
- substrate can be easily passed through the enzyme several times

There is a useful table of advantages and disadvantages of immobilisation near the bottom of the document at http://www.biologymad.com/master.html?http://www.biologymad.com/Biotechnology/Biotechnology.htm and a useful summary at http://www.rohmhaas.com/ionexchange/Pharmaceuticals/enzymes.htm and http://www.lsbu.ac.uk/biology/enztech/imeconom.html

The use of dipsticks and biosensors in the quantitative measurement of glucose

Glucose is not usually detectable in the urine of healthy mammals – although it is filtered out of the blood in the kidney, it is reabsorbed in the proximal tubules. Humans with diabetes are unable to control the levels of glucose in their blood and their urine will usually contain variable amounts. One way to determine the quantity of insulin needed is to test a sample of urine with a glucose dipstick.

The dipstick has the enzyme glucose oxidase on its surface – this oxidises the glucose into hydrogen peroxide and gluconic acid. The hydrogen peroxide will oxidise an indicator chemical on the dipstick, which changes colour. The exact colour change is related to the concentration of glucose in the urine sample. The colour can be compared with a colour chart to give a reading for the glucose concentration.

There is a nicely illustrated summary of this in http://www.irvingcrowley.com/cls/urin.htm

It is now more common to measure the glucose concentration of the blood, using a glucose biosensor. A biosensor is a device which uses a biological material, such as an enzyme, a cell or an antibody to detect or measure a chemical compound. The reaction between the biological material and the chemical being measured brings about a change which is converted to an electrical signal by an appropriate

transducer. The electrical signal is then amplified to give a read-out on a digital display.

The glucose biosensor works as follows :

- It contains a layer of immobilised glucose oxidase enzyme.
- This enzyme binds with any glucose in the blood, which is oxidised with dissolved oxygen from the solution to form hydrogen peroxide and gluconic acid.
- An electrode (usually platinum) measures the drop in oxygen concentration as it is used to make hydrogen peroxide. The electrode generates an electrical signal.
- The size of the electrical signal is proportional to the concentration of glucose in the blood.
- A digital readout gives the user a figure for the glucose concentration

http://www.ul.ie/elements/Issue6/Biosensors-%20Elements.htm
The first part of this is about glucose biosensors http://www.chemsoc.org/chembytes/ezine/1998/palmer.htm
This university practical protocol gives interesting insights into the practicalities of using biosensors to measure glucose concentrations http://www.lsbu.ac.uk/biology/enzyme/practical5.html

The production of monoclonal antibodies, using the hybridoma method

Monoclonal antibodies are obtained from clones of single B cells. Unfortunately, B cells will not grow in culture and this problem has to be got round by fusing them with malignant B myeloma cells i.e. cancerous B cells. Myeloma cells will continue to grow and divide indefinitely, though they do not produce antibodies. The fused cells produced from myeloma cells with B cells are known as *hybridomas*. The hybridoma cells will also continue to grow and divide (given suitable and adequate nutrients) and they do secrete antibodies. The antibodies that they secrete are the specific antibodies that were produced by the original clone of B cells.

The production of monoclonal antibodies involves the following stages :

- A mouse is injected with antigen for which the antibodies are required
- An immune response takes place and the mouse plasma cells start to make the antibody
- Plasma cells are extracted from the mouse
- The plasma cells are fused with B cell myeloma cells
- The resulting hybridoma cells are separated individually and allowed to grow, divide and produce antibodies
- Some antibodies are removed and tested with the relevant antigen, to make sure they are the correct monclonals
- Those hybridoma cells which are producing the required antibodies are cultured in a large fermenter
- The monoclonal antibodies are harvested and purified

http://www.accessexcellence.org/RC/AB/IE/Monoclonal_Antibody.html (follow link for illustration in Graphics Gallery)
http://www.vetmed.ucdavis.edu/Animal_Alternatives/mabs.htm
Here is a virtual lab simulation of using monoclonal antibodies in diagnosis – click to enter lab
http://www.hhmi.org/biointeractive/immunology/vlab.html

The use of monoclonal antibodies in the diagnosis and treatment of disease and pregnancy testing

Diagnosis – because the monoclonal antibodies produced from a clone of B cells are all identical, they can be used to identify macromolecules with a very high degree of specificity. For example, they are now routinely used for the following:

- Blood typing before transfusions
- Tissue typing before transplants
- Identification of pathogens – using monoclonals, it is now possible to distinguish between different strains of certain pathogens, which would, otherwise, be every difficult
- Identification and location of tumours
- Detection of HIV
- Distinguishing between different types of leukaemia

This website gives a clue as the variety of monoclonal antibodies available for various applications
http://www.antibodyresource.com/findantibody.html

Treatment – principally, there are two ways in which monoclonal antibodies are used in the treatment of disease:

- Production of passive vaccines – monoclonal antibodies can be injected directly into the blood to attack a particular pathogen
- 'Magic bullets' – monoclonal antibodies can be produced which will combine specifically with cancer cells. It is now possible to bond cancer drugs to such antibodies. In this way, the drugs can be delivered directly to the tumour, thereby reducing the risk of damaging healthy cells.

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

Pregnancy testing – soon after becoming pregnant, women produce a hormone, called *human chorionic gonadotrophin (HGC).* This hormone is produced by the placenta, so can only be present during pregnancy. Monoclonal antibodies are now used to detect the presence of this hormone in the urine – such a pregnancy test can be done very quickly and easily.

This type of pregnancy testing kits work as follows:

- The kit consists of a 'sampler', which is a type of dipstick with an absorbent pad
- On the surface of the pad are monoclonal antibodies, specific to HGC and to which coloured latex particles are attached – when the pad is moistened, the molecules of the antibody begin to move
- The sampler is dipped into urine – if HGC is present, it will bind to the monoclonal antibodies and will be drawn up the pad
- Further up the pad is an area at which there is a line of immobilised HCG antibodies

- Any HCG molecules drawn up the pad will bind with these antibodies and the latex particles will create a coloured line. This is a positive result.
- Further along the pad is a second line of immobilised antibodies, to which will bind any HCG antibodies without HCG. A coloured line in this second area (but no coloured line in the first area) will confirm that the HCG antibodies have moved up the pad, but that the result is negative.

General information about pregnancy test kits http://www.madehow.com/Volume-4/Home-Pregnancy-Test.html
Information about one particular pregnancy test kit http://www.xpressdrugtest.com/preg.html

Biotechnology Self Assessment Questions

SAQ 1 Outline the properties of some bacteria which make them suitable for use in the extraction of heavy metals from their ores.

SAQ 2 In the following table, list some of the advantages and disadvantages of batch culture when compared with continuous culture.

advantages of batch culture	disadvantages of batch culture

SAQ 3 Explain how a population of bacteria might develop antibiotic resistance.

SAQ 4 With reference to enzyme immobilisation :

(a) Describe how an enzyme is immobilised in alginate.

(b) Outline the advantages of using immobilised enzymes, rather than enzymes that have not been immobilised.

SAQ 5 Explain what is meant by a biosensor and outline the working of a glucose biosensor.

SAQ 6 With reference to Monoclonal Antibodies :

(a) Outline the use of hybridomas in their production.

(b) Explain their use in the diagnosis and treatment of disease.

CROP PLANTS

Key Objectives

- To be able to describe and explain structural features of a named, wind pollinated plant
- To compare the genetic outcomes of self and cross-pollination
- To be able to describe the structure of the fruit in maize and explain the function of the endosperm
- To be able to explain the significance of the inclusion of the grains of cereal crops in the human diet
- To be able to explain how the leaves of C_4 plants (such as maize or sorghum) are adapted for high rates of carbon fixation at high temperatures
- To be able to explain how sorghum is adapted to survive in arid environments
- To be able to explain how rice is adapted to survive with its roots submerged in water
- To be able to explain various examples of how crop plants can be improved. :

    Hybridisation to produce polyploids in wheat

    Inbreeding and hybridisation to produce vigorous, uniform maize

    Genetic manipulation to enhance the vitamin A content of rice

Key Definitions

- *Pollination* - the transfer of pollen from an anther to a stigma.

Self-pollination is when the transfer occurs in the same flower or from one flower to another on the same plant.

Cross-pollination is when the transfer is from one plant to another.

- *Xerophyte* – a plant which has a number of structural and physiological features which allow it to live successfully in areas of low water supply i.e. arid environments.
- *Hybrid* – a plant which is the result of interbreeding between two different species.
- *Polyploid* – a plant which has more than two sets of chromosomes.

Key Ideas

The structural features of a wind pollinated plant

Wind pollinated plants have flowers with a number of structural features, which distinguish the from insect pollinated plants. These include:

- Flowers are borne at the end of long stalks, held well above the foliage. In some cases, the flowers appear before the leaves.
- Small, inconspicuous petals – often green in colour. Petals may be absent altogether.
- Stigmas are large, branched and feathery – and held outside the flower.
- Stamens are pendulous and also hang outside the flower.
- Anthers are versatile i.e. they are attached at the midpoint, so they will swing freely in the wind.
- Pollen grains are relatively light and small.
- Pollen grains are produced in very large quantities.
- Absence of nectaries.
- Absence of scent.

http://en.wikipedia.org/wiki/Anemophily http://florawww.eeb.uconn.edu/collections/pollination.html

The genetic outcomes of self and cross pollination

Assuming that pollination results in successful fertilisation, the main genetic outcomes will be as follows:

self pollination	cross pollination
decreased genetic variation / increased genetic uniformity	increased genetic variation / decreased genetic uniformity
increased homozygosity / decreased heterozygosity	increased heterozygosity / decreased homozygosity
harmful recessive characteristics more likely to be expressed	harmful recessive characteristics less likely to be expressed
reduction in gene pool	maintenance of gene pool
inbreeding depression reduces fitness	gives hybrid vigour (= heterosis = outbreeding enhancement), so fitness maintained

All of these outcomes mean that populations which result largely from cross pollination are phenotypically more variable, which gives them more evolutionary potential and means that they are better able to adapt to changes in the habitat or environment.

http://en.wikipedia.org/wiki/Heterosis http://en.wikipedia.org/wiki/Inbreeding_depression

The structure of the fruit in maize and the function of the endosperm

The individual fruit of maize is a dry fruit (known, botanically, as a *caryopsis*) and contains a single seed.

The seed contains two structures – a *germ,* from which a new plant will develop and an *endosperm*, a store of nutrients which will be made available to the germinating seedling until it has established sufficient leaf area to photosynthesise.

The germ consists of a miniature plant axis to which are attached around five embryonic leaves and a radicle, from which the root will develop. The germ is the source of maize 'vegetable oil'.

The endosperm takes up about two thirds of the volume of the seed and accounts for around 86% of is dry weight. The principal component of the endosperm is starch, together with about 10% protein (gluten). Its function is to provide the nutrition required by the germinating seedling – though it is also the basis of the nutritional value of maize. Whole, ground maize meal has an energetic value of around 1500 kj per 100g.

http://www.science.siu.edu/plant-biology/PLB117/Nickrent.Lecs/Fruits.html, click on 0535.jpg for an image of a corn grain. http://quorumsensing.ifas.ufl.edu/HCS200/Seed.html has protocols for investigating a number of fruits including a maize caryopsis.

The significance of the inclusion of cereal grains in the human diet

Cereal grains are a major component of the human diet in many parts of the world. In terms of nutritional value, most cereal grains are similar though they do vary in the levels of some nutrients, such as vitamins and minerals. The final nutritional value will also depend greatly on the amount of processing involved.

The nutritional value of cereal grains include:

- Carbohydrates (mainly starch) are a major component of cereals – usually 70 to 80%. Hence, they are a very important source of energy.
- Source of protein. Most cereals have a protein content of between 6 and 14%. In general, millets, rice and maize are at the low end of the protein range, rye and barley are intermediate and wheat and oats are high.
  The main problem with regard to the provision of protein is that cereals do not always provide a balance of amino acids. They are especially low in lysine, an essential amino acid, which means that other sources of this need to be included in a balanced diet.
- All cereal grains are low in fat. This is usually 2 – 4%, though oats are an exception with 7.5%. However, they are high in essential fatty acids, such as linoleic acid.
  Most of the fat is found in the germ of the grain.
- Vitamins – all cereals provide a good source of the B group vitamins (thiamin, riboflavin, niacin, B6, folic acid, biotin and pantohenic acid) and the fat soluble vitamin E. However, they are deficient in vitamins A, D and C.
- Minerals – a range of minerals are found in most cereals. These include potassium, calcium, magnesium, phosphorus, iron and zinc.
- Fibre – cereals are an excellent source of dietary fibre. Wholegrain meal or flour will contain much more fibre than grains which have been processed and refined.

http://www.food.rdg.ac.uk/online/fs552/Cereals.ppt reviews the nutritional importance of some key cereals.
http://en.wikipedia.org/wiki/Cereal

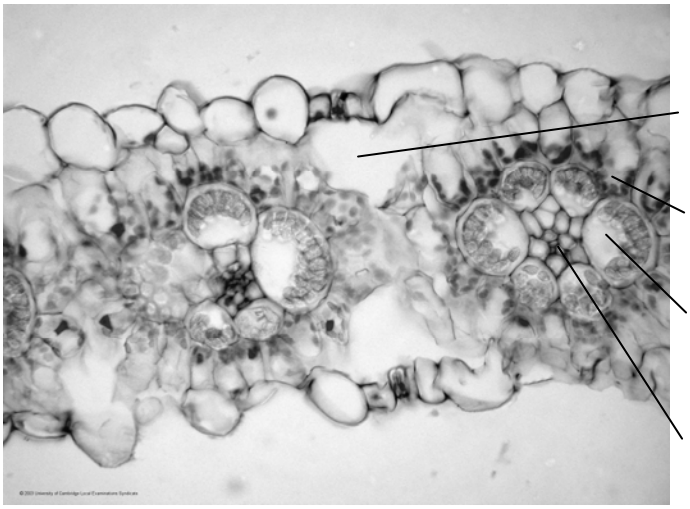
Adaptations of the leaves in C_4 plants

Conditions of high temperature and high light intensity will increase the rate of photorespiration in plants. In photorespiration, the enzyme ribulose bisphosphate carboxylase (Rubisco) acts as the catalyst for the combination of oxygen with RuBP, instead of carbon dioxide. This results in an overall intake of oxygen and output of carbon dioxide and means that less RuBP is available for carbon dioxide fixation.

Conditions which promote photorespiration are found in the tropics. Tropical grasses have a leaf structure which allows them to avoid photorespiration. Such plants are referred to as C_4 plants. Some of the most productive crop plants in the world are C_4 plants - for example, sugarcane and maize.

The structural features of the leaves which distinguish C_4 plants are as follows :

- Around the vascular bundles are arranged a group of cells known as *bundle sheath cells.* These cells contain RuBP and Rubisco, but have no direct contact with the air and, therefore, are not exposed to high concentrations of oxygen.
- Around the bundle sheath cells is another ring of *mesophyll cells* – these are in contact with air spaces, but have no air spaces between them, ensuring that no oxygen reaches the bundle sheath cells.
- The mesophyll cells contain an enzyme called *PEP carboxylase*, which catalyses the combination of carbon dioxide with a compound called phosphoenolpruvate or PEP. This results in the formation of oxaloacetate.
- This oxaloacetate is then converted to malate, which is passed on to the bundle sheath cells, where carbon dioxide is removed from the malate and combined with RuBP in the usual way. The Calvin cycle then proceeds as normal.



maize leaf t.s, high-power (x500)

air spaces well away from bundle sheath cells, beneath stomata

mesophyll cells with chloroplasts, but no air spaces between them

bundle sheath cells, Rubisco is isolated from oxygen in the air so that photorespiration is minimised

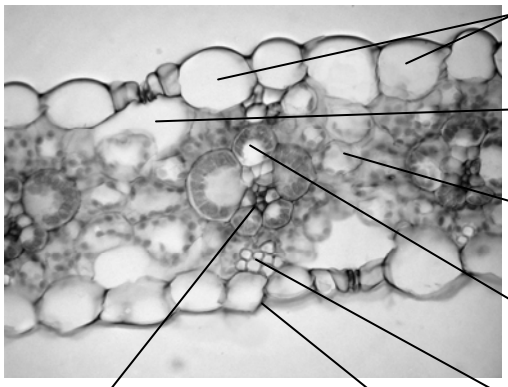
vascular bundle containing xylem to supply water and phloem to translocate sucrose away from the leaf

How sorghum is adapted to survive in arid environments

Sorghum is the fifth most important cereal crop in the world. It is of particular importance in areas of low rainfall – it is able to grow successfully in areas of low water supply and shows many of the characteristics associated with *xerophytes* (plants adapted to living in arid conditions). These include :

- A very dense root system – both widespread and deep, allowing an efficient uptake of whatever water is available.
- The leaves are covered with a thick, waxy cuticle especially on the lower surface – reduces the evaporation of water from the surface of the leaves.
- Specialised *motor cells* (bulliform cells) on the upper-side of the leaves and strengthening tissue (sclerenchyma) below the vascular bundles, which cause the leaves to roll inwards when water is in short supply, hiding away half of the stomata. This allows the build up of water vapour, reducing the difference between the water potential inside the leaf, again reducing the diffusion of water vapour from the leaves.
- The number of stomata is low and the air spaces inside the leaf are small. They are only found well away from the vascular tissues, increasing the distance that the water has to diffuse before it is lost from the leaf.
- Like maize, it is a C4 plant, so, as long as there is sufficient water, sorghum can continue to photosynthesise even when it is very hot and sunny.

sorghum leaf t.s, high-power (x500)



motor cells (bulliform cells) which cause the leaf to roll up when they lose turgor, as a result of drought

small air spaces well away from bundle sheath cells, close to the few stomata

mesophyll cells with chloroplasts, but no air spaces between them

bundle sheath cells, Rubisco is isolated from oxygen in the air so that photorespiration is minimised

vascular bundle containing xylem to supply water and phloem to translocate sucrose away from the leaf

strengthening tissue (sclerenchyma) to support leaf blade when it wilts and rolls up

thick cuticle on lower surface of leaf

How rice is adapted to grow with its roots submerged in water

Key varieties of rice may be described as ‘swamp plants’. As crop plant, it is often grown partly submerged in paddy fields. Fields are flooded, then ploughed and the young rice plants are planted in the resulting mud. Oxygen levels in the mud fall very rapidly as the oxygen is used up by respiration of bacteria in the mud – and levels remain very low in the flooded paddy fields since oxygen can only diffuse very slowly through the water.

Rice plants have a number of adaptations which allow them to grow successfully in these conditions of low oxygen availability:

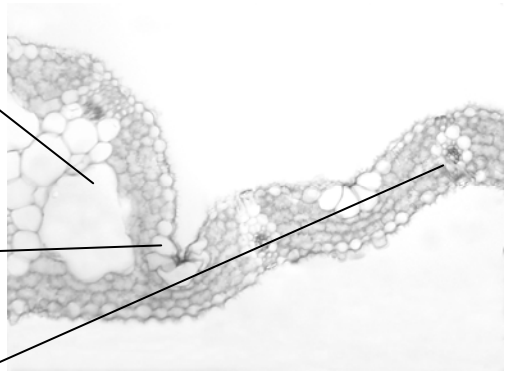
- The stems and leaves possess very large air spaces, running the length of the stem – these allow oxygen to get through to the roots from the air.
- The roots are very shallow – this allows them some access to the higher levels of oxygen in the surface water.
- When oxygen concentrations fall to very low levels, the roots are able to respire anaerobically. This results in the production of alcohol, which would normally be toxic. However, rice root cells show an unusually high tolerance to alcohol - they are able to produce high levels of the enzyme *alcohol dehydrogenase*.

rice leaf t.s, medium-power (x200)

air spaces that run right down through the leaves and stems into the roots

motor cells (bulliform cells) which cause the leaf to roll up when they lose turgor, so that the rice is protected in case the fields dry out

C4 organisation so Rubisco is isolated from oxygen in the air so that photorespiration is minimised in hot conditions



http://www-plb.ucdavis.edu/labs/rost/Rice/Roots/rtgro.html http://www.biologymad.com/resources/Crop%20Plants.pps

Producing polyploids in wheat through hybridisation

Modern bread wheat (*Triticum aestivum*) is a hexaploid plant i.e. it possesses six sets of chromosomes (6n). It is the result of the hybidisation of several wild species of grasses. These species are closely related, so their chromosome numbers and structures are similar but not identical. Such inter-specific hybrids contain one set of chromosomes from one parent species and a second, non-homologous, set of chromosomes from the other, different, parent species. When they attempt to undergo meiosis, it fails because the chromosomes cannot line up with their homologous partner chromosome. Such sterile hybrids could only reproduce asexually.

However, in all organisms, including these sterile hybrids, occasional errors occur in the cell division during the formation of gametes, so that some gametes are diploid (2n) rather than haploid (n). The chromosome number of such gametes is doubled as a result of rare failures of the chromosomes to separate during a mitotic division (termed non-disjunction) before or during gamete formation. If such diploid (2n) male gametes fuse with diploid (2n) female gametes, for example after self-pollination, the resulting offspring is tetraploid (4n). The tetraploid plant contains two sets of chromosomes from each of the original parent species, so there *are* homologous pairs of chromosomes. Meiosis can occur normally as there are now homologous

pairs of chromosomes, and so a new, fertile, species of plant has been instantly formed.

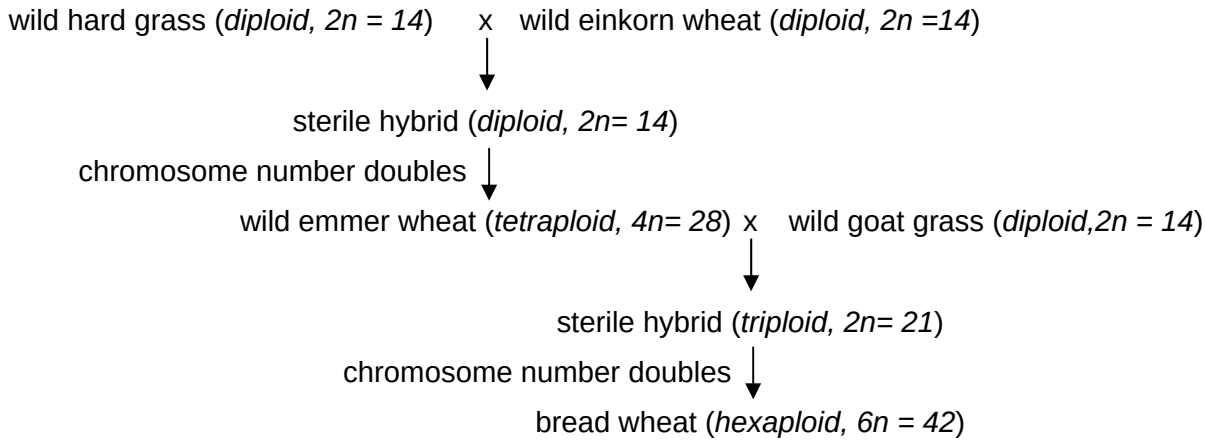
The doubling of the chromosome number appears to have occurred twice during the evolution of modern wheat – resulting in the formation of fertile polyploids from previously sterile hybrids. It is thought that the first doubling of the chromosome number occurred about 0.5 million years ago and the second about 9,000 years ago.

The formation of polyploids has been important in the evolution of plant species – though less important in animals as animal polyploids are often not viable.

It is now possible to induce the formation of polyploids by preventing spindle formation, using chemicals such as colchicine.

Among plant species, polyploids are generally more hardy and higher yielding than their parent species – making them important food crops. The ancestors of wheat are small, not very robust, and produce small ears of small seeds, in contrast to modern hexaploid (6n) wheat.

The following diagram summarises the evolution of modern bread wheat :



http://users.rcn.com/jkimball.ma.ultranet/BiologyPages/P/Polyploidy.html http://en.wikipedia.org/wiki/Polyploidy

Producing vigorous, uniform maize through inbreeding and hybridisation

Maize is one of the most widely grown crop plants. Growing conditions will vary considerably in different parts of the world (soil type, prevailing temperature, rainfall etc) – hence, through selection, inbreeding and hybridisation, growers have been able to produce varieties that grow vigorously (and are, therefore, high yielding) and uniformly under the prevailing conditions. Assuming that conditions remain similar year after year, farmers can continue to grow the same variety and expect to obtain a similar crop.

The characteristics which are desirable in a crop plant such as maize are:

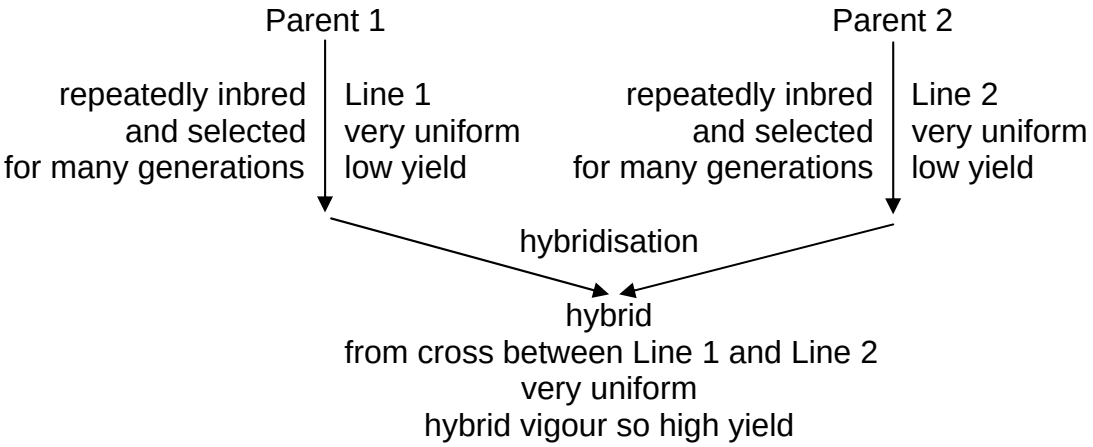
- high yielding
- disease resistant

- good quality in terms of desirability to market
- vigorous growth under the prevailing conditions.
- plants all grow to a similar height (making harvesting easier)
- crops are all ready to harvest at the same time

Given the normal range of genetic and phenotypic variation in a population of plants, when maize was fist cultivated, most plants would only show some of these characteristics. Plants which did show some of the desirable characteristics would be selected and self pollinated. When pollen has been transferred to the stigma, a muslin bag is placed around the flower to ensure that pollen from other plants does not reach the stigma. If this is repeated for many generations, plants will be produced which are homozygous for the desired characteristics.

Maize is a natural outbreeder and it is not very tolerant of inbreeding – such inbreeding can lead to a loss of vigour and fertility, as well as a reduction in size and yield. This is known as *inbreeding depression*. However, the inbred maize has very little variation, with every plant having the same alleles of every gene.

However, if two inbred lines are crossed, it will produce a hybrid that has a greater yield and is more vigorous than either of the parental lines. This is known as *hybrid vigour*. This hybrid is heterozygous for most genes, so deleterious recessive alleles are hidden, but at the same time it inherits the lack of variability from its parents. Such single cross hybridisation has been used for selective breeding since the early 1960's to double the yield (from 4 → 8 tonnes per hectare) and to breed uniform, high yielding maize.



About 100 years ago maize breeding, the first attempts at using inbreeding for maize breeding began. Initially the inbred lines were so low yielding that too little seed was available for the market. For this reason, double crosses were used. Two inbred lines were crossed to create a hybrid, which was then crossed with an unrelated hybrid from two other inbred lines. The resulting double cross hybrid was rather more variable, but more seed was made available. Double cross hybridisation as a selective breeding tool increased maize yields between 1920 and 1960 from 1.5 to 4 tonnes per hectare.

In order to carry out the inbreeding or to carry out a cross to form a hybrid, pollen from a specific male parent must be used to fertilise a specific female parent. To

ensure that the cross intended is the only one that occurs, anthers are removed from some flowers which will form the female parent. Pollen is transferred from the anthers of the male parent flowers to the stigmas of the flowers without anthers. Muslin bags are then placed around the fertilised flowers to prevent pollination by any other pollen.

Selection for measurable characteristics such as yield is done by measuring the characteristic and choosing from breeding those plants that express it most strongly, e.g. having the highest yield. Selection for disease or pest resistance is done by exposing the plants to the disease or pest, which kills any that are not resistant

Seeds which result from such breeding are grown and plants showing the desirable characteristics are bred again – the process can be repeated for many generations.

http://res2.agr.ca/CRECO/zea/zea01_e.htm http://www2.mpiz-koeln.mpg.de/pr/garten/schau/ZeamaysL./Maize.html
http://www.genetics.org/cgi/content/full/148/3/923 http://maizeandgenetics.tamu.edu/hybridvigor.htm
http://www.pioneer.com/usa/research/pipeline/articles/improving_products.htm

Enhancing the vitamin A content of rice through genetic manipulation

The green parts of rice plants contain beta-carotene, which is a vital precursor of vitamin A. However, there is no beta-carotene in the grains and n those pars of the world where rice is the principal staple food, small children are very prone to Vitamin A deficiency.

Vitamin A is essential for the operation of the body's immune system and a deficiency causes increased risk of infection, night-blindness and, in some cases, total blindness. Over 1 million children die every year as a result of vitamin A deficiency.

A genetically-modified strain of rice has now been produced which stores significant levels of beta-carotene in the grains. This strain is known as' golden rice' and contains genes which have been transferred from the daffodil and a bacterium. It is suggested that this genetically modified rice contains sufficient beta-carotene to satisfy daily vitamin A requirements with 300 g of cooked rice.

The method generally used to transfer genes into plant cells is to incorporate the genes into a bacterial plasmid and use bacteria such as Agrobacterium tumefaciens to carry the genes into the plant cells. However, in the case of rice (and other crop plants, such as maize) a method has been developed whereby the genes are delivered directly into the cells using small µm-sized tungsten or gold bullets coated with DNA. The bullets are fired from a device that works similar to a shotgun. This delivery device is known as a 'gene gun' and is now a common method used in the genetic transformation of rice.

When this strain of golden rice was first developed, it was thought by some that it would provide an instant solution to the problems of vitamin A deficiency. However, not everyone believes it is the best answer. Given the controversy and concern about GM crops, it still has not been grown in field trials in Asia – and many agricultural experts and environmental groups believe the solution is not to go down the route of

GM crops, but rather to aim for a more balance diet, which would include more fresh vegetables, which have a naturally high content of beta-carotene.

Information about golden rice on the internet tends to be a bit inaccessible and difficult to use. There's some interesting evaluative material in http://www.foe.org/safefood/rice.html that might prove useful. Another version of the rice is described at http://www.eufic.org/gb/food/pag/food17/food174.htm and http://www.agbioworld.org/biotech-info/topics/goldenrice/gmgolden.html in which far more carotene is produced.

Crop Plants Self Assessment Questions

SAQ 1 State **four** features associated with wind-pollinated flowers.

1
2
3
4

SAQ 2 Compare the genetic outcomes of self pollination with cross pollination.

self	cross

SAQ 3 Discuss the benefits of including cereal grains in the human diet.

SAQ 4 With reference to photosynthesis:

(a) State the problems faced by plants growing in conditions of high temperature and light intensity.

(b) Explain how C_4 plants overcome these problems.

SAQ 5 Sorghum is an example of a xerophyte. Describe the features of Sorghum which allow it to grow successfully in arid environments.

SAQ 6 Outline how bread wheat, which is hexaploid, has evolved from two original diploid grasses.

SAQ 7 Explain how inbreeding and hybridisation have been used to develop vigorous, uniform maize.

SAQ 8 With reference to rice,

(a) Explain why many children show a deficiency of Vitamin A in areas where rice is a principal component of the diet.

(b) Discuss how rice may be genetically manipulated to help overcome this Vitamin A deficiency.

ASPECTS OF HUMAN REPRODUCTION

Key Objectives

- To be able to describe the histology of the mammalian ovary and testis
- To be able to outline gametogenesis in the human male and female
- To be able to explain the role of hormones in maintenance of the human menstrual cycle and link this to changes in the ovary and uterus during the cycle
- To be able to outline the biological basis of oestrogen / progesterone contraceptive pills
- To be able to discuss and evaluate the biological, social and ethical implications of the use of contraception
- To be able to outline the technique of in-vitro fertilisation (IVF) and discuss its ethical considerations

Key Definitions

- *Gametogenesis* – the production of gametes. More specifically, the production of sperm in the testes is called *spermatogenesis,* while the production of eggs or ova in the ovaries is called *oogenesis*.
- *Mitosis* – a type of nuclear division. During the process, a single cell divides to form two cells, which have the same number of chromosomes and are genetically identical, both to each other and the parent cell. It is the type of division used in growth, repair and asexual reproduction.
- *Meiosis* – the type of nuclear division involved in gametogenesis. A single cell divides twice, to form four cells, which are all genetically different and contain only half the number of chromosomes of the parent cell. For this reason, it is referred to as a *reduction division.*
- *Hormone* – a chemical messenger. Produced in endocrine (ductless) glands, hormones are released into the blood and travel to other parts of the body where they have an affect on specific target cells.

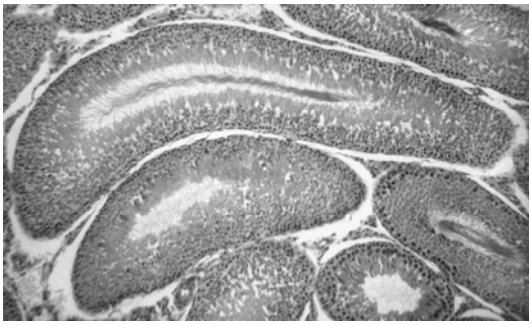
Key Ideas

The histology of the mammalian ovary and testis and Gametogenesis in humans

Spermatogenesis – the production of sperm takes place in the testes. The process begins between the ages of 11 and 15 and will continue for life. Between 100 and 200 million sperm will normally be made every day.

There are a number of stages in the production of sperm:

- Attached to the epithelial layer of the seminiferous tubules are cells called *spermatogonia.* These cells are diploid and divide by mitosis to form more spermatogonia.
- Some spermatogonia move towards the lumen of the seminiferous tubule and increase in size – at this stage, they are called *primary spermatocytes.*
- The primary spermatocytes divide by meiosis. After the first meiotic division, two haploid cells are formed – the *secondary spermatocytes.*
- The secondary spermatocytes undergo a second meiotic division, giving a total of four haploid *spermatids.*
- Each spermatid will mature into a spermatozoon.
- The developing sperm are nourished by *Sertoli cells.*
- The whole process takes approximately 64 days.



medium-power photomicrograph of mammalian seminiferous tubules (X100)

it is important to be able to explain why the tubules, which are all circular in cross-section, appear a variety of different shapes

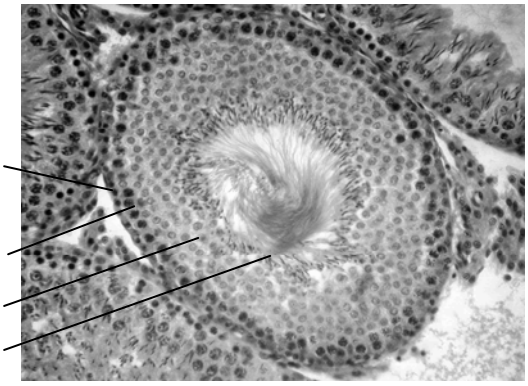
high-power photomicrograph of t.s mammalian seminiferous tubule

layer of spermatogonia attached to epithelium and dividing by mitosis

primary spermatocytes dividing by meiosis and moving towards lumen

haploid spermatids maturing into spermatozoa

mature spermatozoa released into lumen



Oogenesis – the production of eggs (or ova) takes place in the ovaries. However, unlike the production of sperm, the process begins very early in the life of the female – indeed, when she is still only an embryo.

The stages involved are:

- About five weeks after the formation of a female embryo, some cells in the tiny developing ovaries start to divide by mitosis, forming diploid *oogonia.*

- When the embryo is 24 weeks old, there will be millions of oogonia in the ovaries.

- Up until about 6 months after birth, the oogonia will begin the first division of meiosis. The resulting cells are called *primary oocytes*. However, they do not complete the division and remain at prophase 1 for many years. Not all the primary oocytes survive and, at puberty, there will be around 400,000 in the ovaries. They are each approximately 20 µm in diameter.

- When the primary oocyte enters prophase 1, some surrounding cells form a layer around it, forming a *promordial follicle.* Some of these then develop into *primary follicles,* with several layers of surrounding cells, called *granulosa cells.* Others cells from yet more surrounding cells, called the *theca* and the granulosa cells secrete a protective layer of glycoproteins, called the *zona pellucida*. The primary follicles will then remain like this until the girl reaches puberty.

- At the onset of puberty, hormones will stimulate the primary follicles to develop into *secondary follicles*. The oocyte enlarges and a fluid-filled cavity develops.

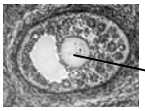
- One primary oocyte begins to grow rapidly, forming a *Graafian* or *ovarian follicle,* which is between 1 and 1.5 cm in diameter.

- The primary oocyte now completes the first meiotic division, producing one large cell and one tiny cell, called the *polar body*. The large, haploid cell is called the secondary oocyte, which continues straight into the second meiotic division. However, it again stops before the division is complete, remaining at the stage of metaphase II.

- The follicle now ruptures and releases the secondary oocyte, surrounded by granulosa cells (ovulation).

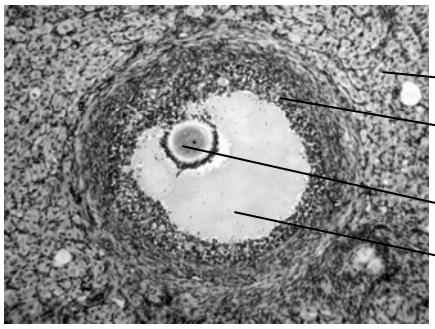
- The oocyte is drawn into the oviduct (fallopian tube), mainly by peristalsis, although there are cilia present in addition. In the oviduct, if fertilisation takes place, it will complete the second division of meiosis.

- The follicle transforms into a corpus luteum.



medium-power photomicrograph of developing follicle (X100)

primary oocyte surrounded by follicle cells and next to fluid-filled cavity, surrounded by ovary tissue



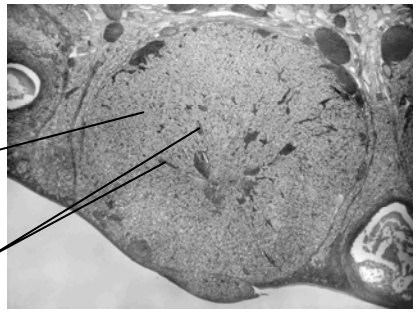
medium-power photomicrograph of mature follicle (X100)

ovary tissue

follicle cells secreting oestrogen

haploid secondary oocyte held at metaphase II of meiosis, ready to be released at ovulation

fluid-filled cavity



low-power photomicrograph of corpus luteum (X50)

corpus luteum tissue that secretes progesterone

some follicle cells within corpus luteum that continue to secrete oestrogen

http://science.tjc.edu/images/reproduction/Index.htm
http://www.columbia.edu/cu/biology/courses/w2501/histology.html
www.visualsunlimited.com Gametogenesis http://users.rcn.com/jkimball.ma.ultranet/BiologyPages/
www.umanitoba.ca/Biology/lab14

The role of hormones in the human menstrual cycle and changes in the ovary and uterus during the cycle

There are a number of hormones involved in the control of the human menstrual cycle.

Two are produced by the ovaries:

- Oestrogen (produced by follicle cells)
- Progesterone (produced by corpus luteum)

Two are secreted by the anterior pituitary gland:

- Luteinising Hormone (LH)
- Follicle Stimulating Hormone (FSH)

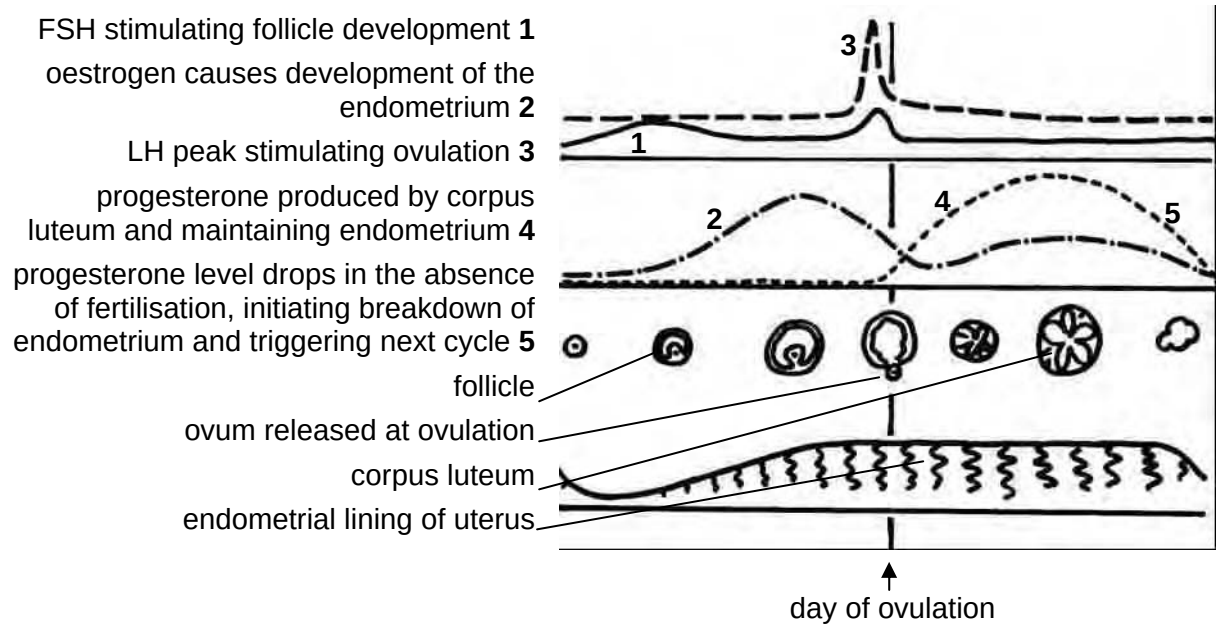
Oestrogen begins to be secreted at the onset of puberty, when brings about an increase in the size of the reproductive organs and the development of secondary sexual characteristics.

The main role of progesterone is to prepare the endometrium (lining of the uterus).

The duration of a normal menstrual cycle is 28 days – day 1 is the first day of menstruation.

The main hormonal changes during the cycle are as follows:

- Secretion of FSH and LH increases slightly during the first few days. This causes a number of primary follicles to develop, though only one in one ovary continues to develop.
- The granulosa cells of the developing follicle secrete oestrogen, the levels of which start to rise sharply during the first half of the cycle.
- The increase in the level of oestrogen inhibits any further secretion of FSH and LH – and their levels start to fall.
- The oestrogen causes the endometrium to thicken – by day 12 it is 3 – 4mm thick.
- Around day 12 the secretion the secretion of LH suddenly rises very sharply. This causes the granulosa cells to reduce their secretion of oestrogen and start to secrete progesterone.
- It also causes ovulation, which normally occurs on day 14 (i.e. half way through the cycle).
- When the egg has been released, the remaining granulosa cells enlarge and fill up with a yellow substance. It is now called a *corpus luteum* or yellow body, which continues to secrete large amounts of progesterone and smaller amounts of oestrogen.
- The progesterone brings about the further development of the endometrium, increasing its supply of blood and the amount of glycogen and lipids in its cells. Its thickness reaches 5 – 6 mm and it is prepared for implantation, if the egg has been fertilised.
- If fertilisation has not occurred, the oestrogen and progesterone inhibit the secretion of FSH and LH from the anterior pituitary gland. As a result, the corpus luteum degenerates.
- All four hormones now fall to a low level and, once the oestrogen and progesterone have dropped far enough, the anterior pituitary again begins to secrete FSH and LH and the cycle begins again.
- The fall in oestrogen and progesterone causes the endometrium to break down and menstruation occurs during the next four to seven days of the cycle.



www.merck.com/mmhe/sec22/ch241/ch241e.html

The biological basis of the oestrogen / progesterone contraceptive pill

The oestrogen / progesterone contraceptive pill is also known as the 'combined oral contraceptive pill' – or, more commonly, as 'the pill'.

As the name suggests, the pill contains oestrogen and progesterone and works by changing the hormone balance of the body, as a result of which ovulation does not take place. Principally, the high levels of oestrogen and progesterone in such combined oral contraceptive pills inhibit the release of FSH and LH from the anterior pituitary gland. This mimics the natural situation during the luteal phase of the menstrual cycle.

In addition, it causes the mucus made by the cervix to thicken, forming a 'mucus plug' in the cervix – this makes it more difficult for the sperm to get through to the uterus. It also makes the lining of the uterus thinner, making it extremely unlikely that a fertilised egg could implant.

As well as being taken orally, the hormones can be administered by injections or by slow release skin patches.

It is a very effective method of contraception.

The biological, social and ethical implications of the use of contraception

Biological implications – methods of contraception which do not involve hormones (e.g. barrier methods etc.) do not really have any biological implications. However, there are many biological implications when using the oestrogen / progesterone pill.

Benefits:

- Reduces the risk of developing certain ovarian cysts
- Reduces the risk of developing cancer of the ovary or uterus
- Menstruation is more regular and it may help to relieve pre-menstrual tension
- Reduces the risk of pelvic infection – the mucus plug may prevent bacteria getting into the uterus

Side effects and possible risks:

- Some women may develop nausea and head aches
- Tiredness and mood changes
- Rise in blood pressure
- Increased risk of thrombosis. In some women this can be very serious – may cause a stroke or a blood clot in the lungs
- Small increased risk of breast cancer

Social implications – in many parts of the world, the use of contraception is referred to as 'Family Planning'. As this suggests, the availability of contraception means that

it is easier to choose when to have children. It is also easier to choose not to have children.

The result is that it is possible to plan families around careers and other considerations, such as financial circumstances. It has also meant that, in some countries, the population size has not increased to the extent that it would have done in the absence of contraception. Indeed, in some areas, there is concern that there are too few children being born to be able to sustain the population in the future.

In those parts of the world where contraception is not freely available, there continue to be problems of overpopulation and the implications that this has for supply of food, water and other resources.

Ethical implications – for many people the benefits of using contraception in terms of control of fertility and birth-control, far outweigh any ethical objections. The benefits may be seen in terms of the opportunities for:

- a woman to decide when and if she will conceive
- countries to control their population growth
- those at medical or psychological risk if pregnant, to avoid such pregnancy
- reduced chance of unplanned pregnancy in sexually active teenagers

Families from many religious groups see birth control as a God-given way of spacing out their pregnancies and maximising the life-chances of their children
For others, ethical objections are seen as outweighing any potential benefits and so some Christians (e.g. Catholics) believe that sexual activity within marriage is God-given for the purpose of reproduction, and so artificial contraception such as the pill is morally wrong

http://www.ivillage.co.uk/health/whealth/birthcontrol/articles/0,,549123_599845-2,00.html
http://en.wikipedia.org/wiki/Contraceptive_pill

In-vitro fertilisation and its ethical implications

In-vitro fertilisation (IVF) is a technique whereby eggs are fertilised outside the woman's body. The process involves controlling ovulation through the administration of hormones, removing eggs from the ovaries and allowing sperm to fertilise them in a fluid medium.

In-vitro is Latin for 'in glass' i.e. it is a reference to fertilisation taking place in glass test tubes. In practice, test tubes are not actually used, but the term is now used to describe any laboratory-based procedure.

The first 'test tube baby' was born in England on July 25th 1978.

The process of in-vitro fertilisation involves the following stages:

- *Ovarian stimulation* – treatment would normally start on the third day of menstruation. This involved the administration of hormones which will have a similar action to FSH and will stimulate the development of multiple follicles in the ovaries. Usually, about 10 days of injections will be necessary.

- *Oocyte retrieval* – when the development of follicles is judged to be adequate (usually by monitoring oestrogen levels), the hormone human chorioic gonadotrophin (HCG) is given. This has a similar effect to Luteinising Hormone and would be expected to cause ovulation about 42 hours after injection. In practice a needle is used to remove eggs directly from the ovaries prior to ovulation taking place.

- *Fertilisation* – the eggs are stripped of any surrounding cells and are incubated with sperm (in a ratio of approx 75,000 : 1) for about 18 hours. By that time fertilisation should have taken place. Where the sperm count is very low, it is now possible to inject a single sperm directly into the egg.

The fertilised egg will now be placed in a special growth medium and left for 48 hours, by which time it should have reached the 6 – 8 cell stage.

- *Embryo transfer* – embryos which are growing successfully are transferred to the patient's uterus through a thin, plastic catheter. Often, several embryos are transferred to improve the chances of implantation and pregnancy. During the next two weeks the woman will be administered progesterone, which will help to keep the uterus lining thickened and suitable for implantation.

The chances of a successful pregnancy using IVF is approx 20 – 30%. There are many factors which can influence the success rate – for example, age of the patient, quality of the eggs and sperm and health of the uterus.

The main complication of IVF is the possibility of multiple births – this is largely due to the practice of transferring several embryos to the uterus at the same time.

The possibility of birth defects is a controversial subject in IVF treatment – though most studies do not show a significant increase compared with normal fertilisation.

When multiple embryos are produced, it is possible to freeze embryos in liquid nitrogen, when they can be preserved for a very long time. The advantage of this is that if the patient failed to conceive frozen embryos can then be used, without the need to go through the full cycle. Also, if pregnancy did occur, they could return later for a second embryo transfer and pregnancy.

Ethical implications of IVF – there are a whole range of ethical issues that may result from the process of IVF. These include:

- Bypassing the natural method of conception and making pregnancy into a technological / medical process
- Expensive so unavailable for many people and may reduce life-chances of children who cost more to create than through natural conception
- Fertilising more embryos than will be needed and then discarding unwanted embryos
- Freezing and long-term storage of embryos with unknown potential effects
- The potential to create embryos for research or to grow tissues and organs for transplant

- The potential to select and modify embryos

In addition to these issues, IVF also allows babies to be created away from the traditional mother-father model. The process requires sperm, eggs and a uterus – potentially, any of these can be provided by a third party, creating additional ethical and legal considerations. It also provides more possibilities for single people and same sex couples to have children. IVF has allowed women to become pregnant after the menopause. Even after menopause, the uterus is able to carry out its function – though the egg would have to come from an egg donor. This would mean that there would be no genetic link between the woman and the child to which she gives birth. People vary in how acceptable they find some of these issues.

For many people, IVF is seen as acceptable, for example, to permit selection of embryos that do not contain lethal alleles of key genes, for example the allele that causes Huntington's disease. People have more difficulty with the potential to select embryos for intelligence, gender or absence of minor defects. Some religious groups, (e.g. catholic Christians) are totally opposed to IVF – sometimes seeing infertility as a call from God to adopt children and may see IVF as usurping the role of God in bringing into the world the children that He wants.

www.biologymad.com http://en.wikipedia.org/wiki/In_vitro_fertilization
http://en.wikipedia.org/wiki/Sexual_reproduction#Reproduction_in_mammals

Aspects of Human Reproduction Self Assessment Questions

SAQ 1 Describe the following processes in humans:

(a) Spermatogenesis

(b) Oogenesis

SAQ 2 With reference to the human menstrual cycle, describe:

(a) the changes in hormone levels during the cycle.

(b) the changes in the ovary and uterus during the cycle.

SAQ 3 With reference to contraception :

(a) explain the biological basis of the oestrogen / progesterone pill

(b) discuss the social and ethical considerations.

SAQ 4 With reference to in-vitro fertilisation:

(a) Describe how the process is carried out.

(b) Discuss the ethical considerations.

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