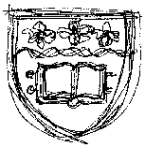
SymbioticA

Biotech Art

Workshop

Presented by

Oron Catts & Gary Cass



University of Wollongong

Contents

SymbioticA Biotech Art Workshop
20-24 June 2005
University of Wollongong

Venues:

Labs – Biological Sciences, University of Wollongong

Seminars – Creative Arts, University of Wollongong Rm 107/ Rm 128 or FCA gallery

Workshop Schedule:

Mon 20 June: lab	10 am	Meet at Faculty of Creative Arts Gallery foyer
	10.30-11 lab	Registration and introductions
	11 -11.30	Lab safety
	11.30-1pm	Bacteria; plating from body and environment. Fungi; culture, identification, structure and slide prep
	1-2pm	Lunch
	2-5pm rm 107	Seminar: Aesthetics and Ethics in biological research and in bioart; speakers: Oron Catts, Dr Anna Munster, Assoc Professor Susan Dodds,
	5-6pm	Informal discussion and de-brief, rm 128, Creative Arts
	* We will send directions to meeting point	
Tue 21 June: lab	10-11.30am	DNA extraction
	11.30-1pm	Set-up Restriction Enzyme digest of pBADgfpuv
	1-2pm	Lunch
	2-5pm	Genetic transformation of pBADgfp Gel electrophoresis of R.E. digest of pBADgfpuv
	5-6pm	Informal discussion and de-brief, rm 128, Creative Arts
Wed 22 June: lab	10-11am	Analysis Gel electrophoresis and discuss Fields of Genes. Results of the pBADgfpuv transformation.
	11-11.30am	Morning tea
	11.30-1pm	Tissue Culture, Tissue Engineering and Stem Cells; discuss
	1-2pm	Lunch
	2-5pm	Mammalian tissue culture and tissue engineering practical
	5-6pm	Informal discussion and de-brief, rm 128, Creative Arts
Thu 23 June: lab	10-11am	Bioart workshop; discussion
	11-11.30am	Morning tea
	11.30-1pm	Discussion, ideas and possible projects
	1-2pm	Lunch
	2-5pm	Plant tissue culture; embryo rescue
	5-6pm	Informal discussion and de-brief, rm 128, Creative Arts
Fri 24 June: (optional)		Field day visiting the zoo, botanical gardens, selective breeding farm (plant/animals), GMO research

Theoretical Background

The Cell:

Cells are generally known as the basic building unit of all living systems (the exemption being some of the viruses). In the 1600's Robert Hooke noticed small cavities in plant material. He named them 'cells', meaning 'little rooms'.

With the advent of microscopy in the 1800's, the cell theory was formulated. It states that all living matter is composed of cells. These cells yield all chemical, energy-yielding and biosynthetic reactions. All cells arise from other cells and the hereditary material contained within is passed from parent to daughter cells.

There are two fundamentally different types of cells, the prokaryotes and the eukaryotes. The prokaryotic cells contain genetic material, DNA (deoxyribonucleic acid) in a large circular single molecule. In the eukaryotic cells, the DNA is lateral complex structures known as chromosomes. Other major differences between these two can be seen in Table1.

	prokaryotes	Animals	Plants
Cell membrane	+	+	+
Cell wall	+ non-cellulose polysaccharide plus protein	-	+ contains cellulose
Nucleus	No nuclear membrane	Has nuclear membrane	Has nuclear membrane
Chromosomes	Single, continuous DNA molecule	Multiple, consisting of DNA and protein	Multiple, consisting of DNA and protein
Endoplasmic Reticulum	-	+	+
Mitochondria	-	+	+
Plastids	-	-	Present in many cell types; chloroplasts in photosynthetic cells
Ribosome	+ (smaller)	+	+
Golgi bodies	-	+	+
Vacuoles	-	Small or absent	Usually large single vacuole in mature cell
Centrioles	-	+	- (in higher plants)

Table 1: A comparison between the prokaryotes and the eukaryotes, plant and animals. (Modified from Curtis, 1983).

The prokaryotes (Fig 1) include the bacteria and the cyan bacteria (once known as blue green algae). Eukaryotes, meaning true nucleus, include fungi, plants and animals. The eukaryotic cells are generally larger and more complex (Fig 1). All multicellular organisms are made up of eukaryotic cells. Some believe that a symbiotic relationship started between a prokaryotic cell and a larger cell giving rise to what we known today as the eukaryotic cell. This new and improved cell had the capability to evolve faster into more complex beings. Mitochondria and chloroplasts are organelles that exhibit similar feature to that found in prokaryotic cells
(Fig 1).

The Nucleus:

Prokaryotic cells do not contain a nucleus, the DNA molecule floats free in the cytoplasm. The DNA of a eukaryotic cell is compartmentalised within a nuclear membrane. The nucleus is a large often spherical structure within the cell containing the hereditary material, the chromosomes. It wasn't till the late 1800's that Oscar Hertwig observed the fertilisation of a sea urchin's egg cell by a single sperm cell. He found that the only link between father and the offspring was the nucleus of the sperm. Today this fact may be in dispute. The belief now being that the sperm cell may contribute other organelles like the mitochondria as well as the DNA.
Walter Flemming around the same time also observed the 'dance of the chromosomes' as a cell divided into two new cells. This process is called mitosis. Mitosis is the method in which the parent cell along with the nucleus divides in two, giving rise to two daughter cells with the same number of chromosomes (same amount of DNA). All cells in this process remain diploid (2n). Meiosis occurs in the gamete cells in which the cell divides further, producing a reduction of chromosome numbers by half. These cells are now termed haploid (n). The fusion of two compatible haploid cells is capable of initiating a new diploid individual cell.

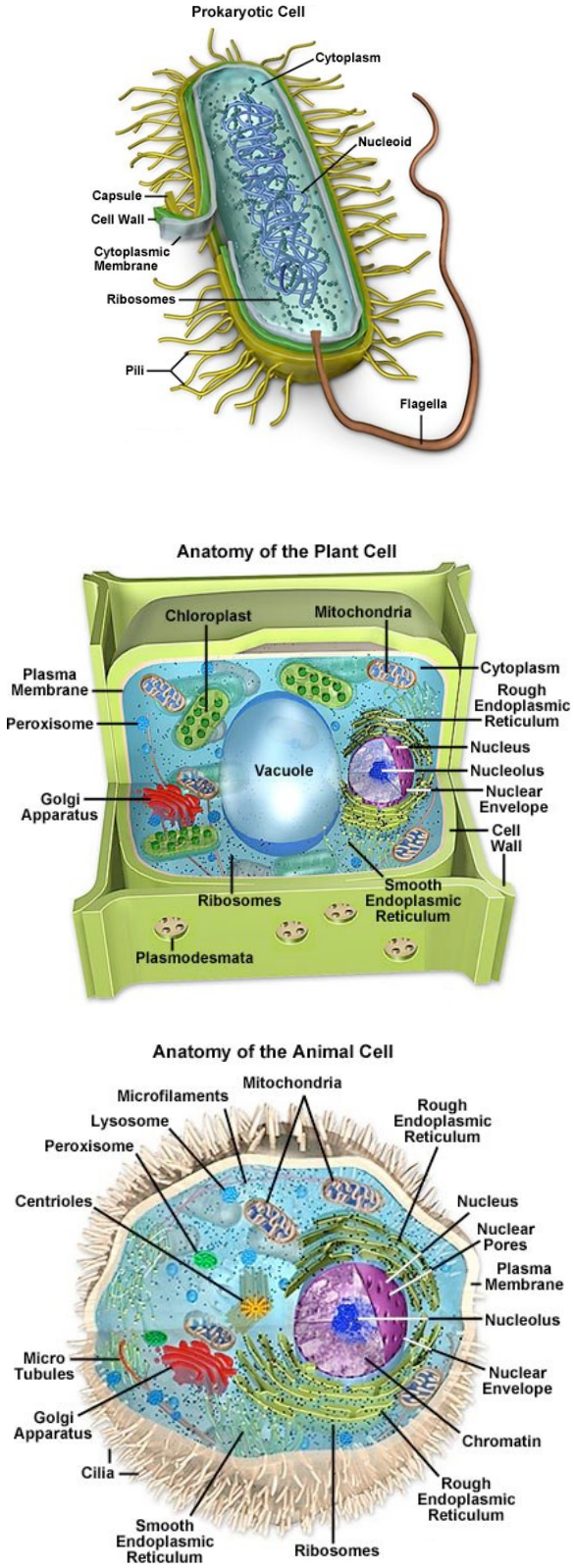


Figure 1: Three drawings showing the cellular structure of the prokaryotic cell and two of the eukaryotic cells, plants and animals. These illustrations also label the organelles generally found in each cell.
Taken from http://www.microscopy.fsu.edu/cells

Genetics:

Genetics is the study of heredity and variability. It goes as far back in history to the beginning of civilisation when humans started to improve crops and/or domestic animals. The ancient Egyptians and Babylonians realised that the male flowers of the date palm had to be brought to the female flowers for the fruit to ripen. Both Plutarch and Lucretius noted the similarities of the children's features to that of their parents. In the late 1600's, Van Leeuwenhoek believed that the human sperm contained 'little men' and the female womb was only used as an incubator.
At about the same time de Graf started another theory, believing it was the female egg that contained the miniature and that the male semen only stimulated the egg's development. By the mid 1800's plant breeders realised that it took a blend of both parents to produce the offspring's characteristics.
At about the same time, Darwin was writing 'The Origin of Species', and an Austrian Monk, Gregor Mendel was working on a set of experiments that would revolutionise genetics. He posed that those discrete factors, now known as 'genes' carried the inherited characters. The genetic makeup of an organism is known as the genotype. These genes also appear in pairs (alleles), one from each parent. By crossing pea plants (parents) with certain visible appearances (phenotype), Mendel could predict the ratio in which the phenotype would be expressed in the offspring (F1 generation). These phenotypic traits are dominant, recessive or codominant.
Most Geneticists, if not the whole biological science community live by the rule;

Phenotype = Genotype + Environment.

Until recently most plant and animal breeders used the Mendelian and related genetic laws in their breeding programmes to select specific desired traits. These so called traditional breeding programmes selected the parents with the desired phenotypes. By crossing the parents, the breeder could try and predict what phenotypic traits would appear in the F1 generation. By carrying on these crosses with other F1 plants and back crosses to the parents, the breeder attempted to generate the desired plant or animal species. Even today these traditional methods are still widely used.

Deoxyribonucleic acid (DNA):

The question still remained, where in the chromosomes are the genes located (knowing that chromosomes contained DNA and protein)? It wasn't till the mid 1900's that scientific proof was found. If we venture back again to the 1800's, 'The Vitalists' believed that the body had to contain a spirit governing heritability. They thought that 'lifeless' chemicals found in the body could not possibly produce a living being. This following was finally quashed by Eduard and Buchner by fermenting alcohol from a substance (enzyme) extracted from yeast. DNA with its simple structure could not possibly control such an intricate system. In 1952 Hershey and Chase experimented with radioactive DNA and proteins to conclude that it was the DNA that contained the genetic material. The following year Watson and Crick presented their model of DNA as a double stranded helical structure, which still holds true today (Fig 2).

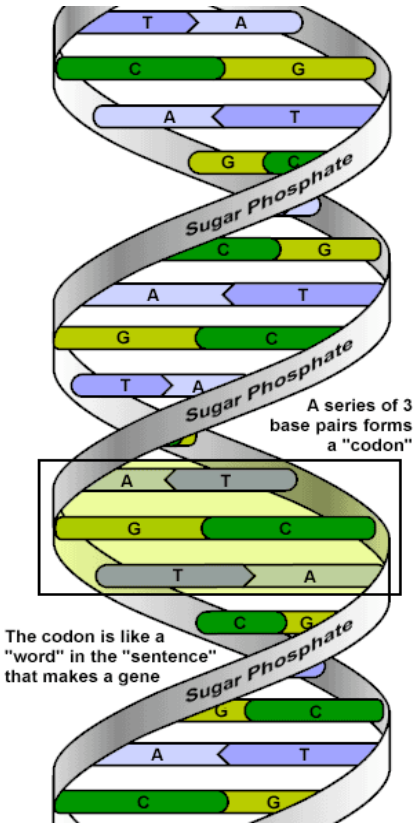


Figure 2: The structure of deoxyribonucleic acid (DNA). Note the base pairing; A's match the T's and the G's with C's.

Taken from http://genetics.gsk.com/overview.htm

Chromosomes are considered to have the following features:

- Long double stranded helix.
- To contain four nitrogenous bases, adenine (A), guanine (G), thymine (T) and cytosine (C). These four letters spell out the genetic code.
- Three bases make up a codon which encodes one or more amino acid.
- That the amount of G's = C's and T's = A's. In DNA, G only pair with C and T only pair with A.
- Each nucleotide contains a sugar (deoxyribose), a phosphate group and a purine (A and G) or pyrimidine (T and C) base.
- At the 5' end the DNA finishes with the phosphate group attached to the fifth carbon on the sugar group.
- At the 3' end the DNA finishes with the phosphate group attached to the third carbon on the sugar group.
- The two strands run in opposite directions. This makes them antiparallel.
- The G bonds to C with three hydrogen bonds.
- The T bonds to A with two hydrogen bonds.

The weak hydrogen bonds that hold the two DNA strands together break easily allowing the strands to separate. This is the first stage of self replication of the DNA. An essential part of heritability is the ability of the DNA to produce exact copies of itself. As each strand separates a new copy is formed along each old strand. Using the old strand as a template, G's pair with C's and T's pair with A's producing two exact copies of the double stranded DNA molecule. A rare occurrence of change in the base pair sequence allows heritable changes (mutations) in the offspring. If the mutation is beneficial for the offspring, then the DNA change is kept. Most mutations are usually fatal.

The DNA language:

Once the structure of DNA was accepted into the scientific community, the race was on to find out how it worked. How did the DNA code for proteins? In 1941, Beadle and Tatum found that one gene coded for one enzyme. They coined the phrase 'one gene – one enzyme', which today has now been upgraded to 'one gene one

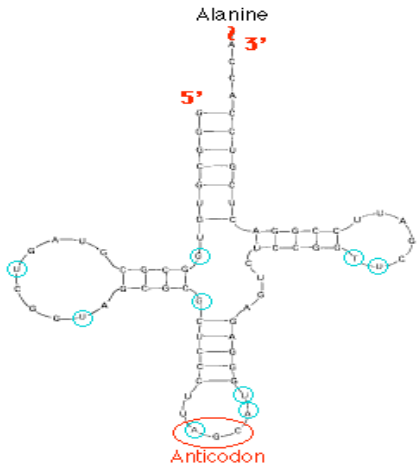
expression'. Note that not all proteins are enzymes, some being hormones such as insulin or structural proteins e.g. collagen.

The genetic code was first broken by Garnow, who stated that the four base pairs in the DNA molecule, G, C, A & T must combine to code for all 20 amino acids. Amino acids being the major components of a proteins structure. If they pair then the possible combinations only code for 16 amino acids, not enough! Therefore it must be triplets of bases, with a possible 64 combinations that code for the amino acids. These triplets became known as codons. Also that one amino acid must be coded by one or more codons (Table 2). But how did the genetic code translate into proteins?

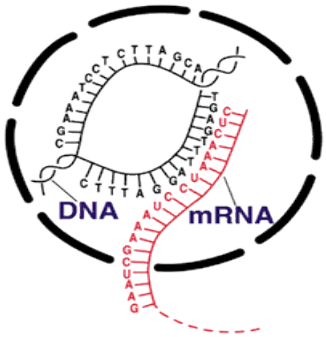
1st base in codon	2nd base in codon: U	C	A	G	3rd base in codon
U	Phe	Ser	Tyr	Cys	**U**
	Phe	Ser	Tyr	Cys	**C**
	Leu	Ser	STOP	STOP	**A**
	Leu	Ser	STOP	Trp	**G**
C	Leu	Pro	His	Arg	**U**
	Leu	Pro	His	Arg	**C**
	Leu	Pro	Gln	Arg	**A**
	Leu	Pro	Gln	Arg	**G**
A	Ile	Thr	Asn	Ser	**U**
	Ile	Thr	Asn	Ser	**C**
	Ile	Thr	Lys	Arg	**A**
	Met	Thr	Lys	Arg	**G**
G	Val	Ala	Asp	Gly	**U**
	Val	Ala	Asp	Gly	**C**
	Val	Ala	Glu	Gly	**A**
	Val	Ala	Glu	Gly	**G**

Table 2: The genetic code

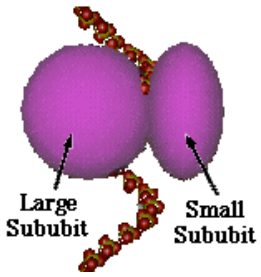
A new molecule came under investigation, ribonucleic acid (RNA). The major differences between DNA and RNA was that RNA contained the sugar ribose and the nitrogenous base thymine (T) was substituted with uracil (U). Also the RNA molecule was single stranded and found mainly in the cytoplasm of the cell. RNA can be found as three differing structural forms (mRNA, tRNA & rRNA), each with a unique role to play in the production of proteins. Messenger RNA (mRNA) was first described by scientists in the 1960's. It was the mRNA that was encoded directly from the DNA via a process termed transcription (Fig4). Once in the cytoplasm, the mRNA is attached to the small subunit of a ribosome (Fig 4). Ribosomes are approximately 2/3 rRNA and 1/3 protein. Each ribosome is made of 2 subunits, a large and small subunit. In the smaller subunit only one type of rRNA occurs. In the large subunit, two types are found. Once the mRNA is attached to the small subunit of the ribosome the larger subunit attaches. Then a tRNA moves into the large subunit (Fig 5). This transfer RNA is a smaller molecule that adapts the DNA code to the protein code (Fig 4). In figure 4 the tRNA at its anticodon site of AGC codes for the amino acid alanine. The anticodon site of the tRNA binds to the appropriate codon of the mRNA. One tRNA has a recognition site specific to one amino acid. The production of a protein starts as the mRNA moves through the small subunit and the codons are converted into a chain of amino acids. This is a process called translation, whereby these chains of amino acids form a highly specific protein. A summary of the entire protein synthesis including transcription and translation can be seen in figure 6.



tRNA coding for alanine**.**



Transcription of **mRNA.**



Ribosome made up of **rRNA** and protein

Figure 4: The structure of ribonucleic acid (RNA). Three different form can be found within the cell, messenger RNA, transfer RNA and ribosomal RNA. Each molecule of RNA completes a highly specific task in the formation of proteins.
The tRNA taken from
http://users.rcn.com/jkimball.ma.ultranet/BiologyPages/T/Translation.html.
The mRNA was taken from
http://homepage.mac.com/yamajinaoki/toub/gouse/gouse.html.
The rRNA was modified from
http://gears.aset.psu.edu/viz/services/projectlist/cell/Text/Gifs/ribosome.gif.

The regulation of this protein synthesis is important for a cell as it may not need all of the proteins at any given time. If it continued to produce all sorts of unnecessary proteins all the time, the cell would expend too much energy. Therefore several tactics have be engaged to regulate the system. One is the rapid degradation of mRNA. Once the mRNA has gone there is no longer a template to read. Another is to deactivate certain proteins. A third is to use a single strand of mRNA to transcribe several genes that all produce enzymes involved in related biochemical reactions. Such a group of related genes is called an operon. The expression of these operons or genes may also be regulated by the presence of a promoter.

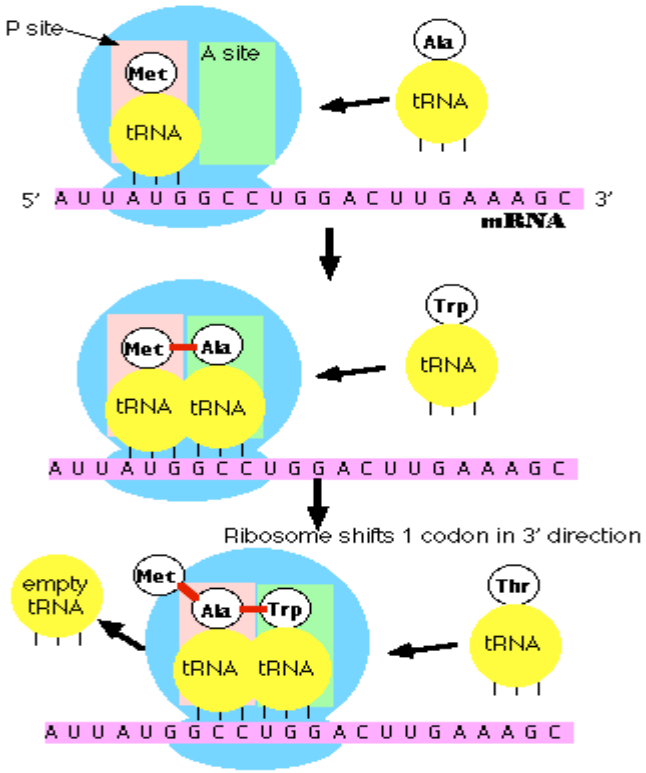


Figure 5: The translation of the mRNA by the tRNA, forming a new chain of amino acids and eventually a highly specific protein.
Taken from
http://users.rcn.com/jkimball.ma.ultranet/BiologyPages/T/Translation.html.

In the pBAD plasmid (the one used in the workshop to transform the bacterial cells), an araC regulator exists. This regulator will only allow the transcription of the genes 'downstream' on the DNA molecule in the presence of arabinose (sugar). Therefore the arabinose can be called the promoter. If present the genes are turned on and if absent the genes are turned off.
The DNA molecule therefore not only contains genes that code for proteins (structural genes) but also the regulatory genes, the on/off switches of the structural genes. Beliefs of today are that much of the so called 'Junk' DNA may be in fact these regulatory genes.

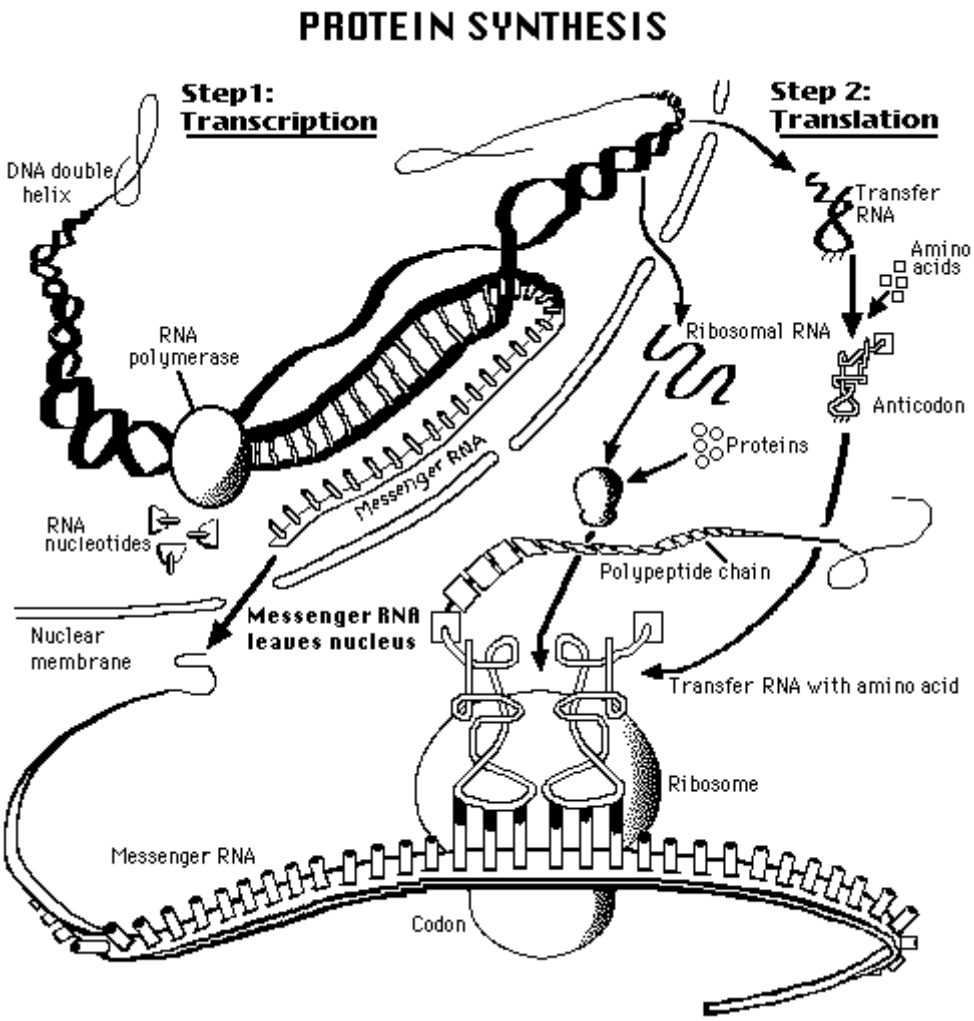


Figure 6: A summary of the complete process of protein synthesis.

Taken from

http://www.emc.maricopa.edu/faculty/farabee/BIOBK/BioBookPROTSYn.html.

Biotechnology:

Encyclopaedia Britannica;

> *Applied biological science (as bioengineering or recombinant DNA technology).*

The Australian Concise Oxford Dictionary 2nd edition;

> The exploitation of biological processes for industrial and other purposes, especially genetic manipulation of micro-organisms (for the production of antibiotics, hormones etc.).

The rapid growth of biotechnology started in the 1970's with scientists developing new techniques in genetic engineering. This growth was speed up by the U.S. Supreme Court decision, ruling that "a live human-made micro-organism is patentable matter". Much of the early pioneering work done in biotechnology used microbes such as the bacteria *Escherichia coli* as well as bacteriophages (bacterial viruses). At this time a smaller molecule of DNA was found in bacterial cells, called plasmids. Plasmids can contain anything from two genes up to as many as thirty genes in its sequence. Many of the genes found in the plasmids coded for resistance to anti-bacterial compounds. Bacterial cells also seem to be able to share these plasmids between each other. Like the bacterial chromosome, the plasmid is circular and self replicating. Figure 7 shows a diagrammatic version of the pBADgfp plasmid that will be used in the workshop. This plasmid is approximately 5400 base pairs in size and contains the 'bla' gene coding for ampicillin resistance. An araBAD operon (group of genes) regulated by the 'araC' gene, is switched on in the presence of arabinose. The 'gfp' gene which codes for the green fluorescent protein has been inserted downstream and will be transcribed (allowing fluorescence) if the araC regulator is stimulated. A full description of the plasmid can be found at the following web address;

www.ncbi.nlm.nih.gov/entrez/viewer.fcgi?db=nucleotide&val=1490531

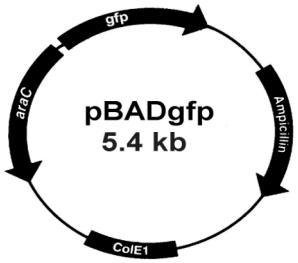


Figure 7: A simplified diagram of the pBADgfp plasmid.

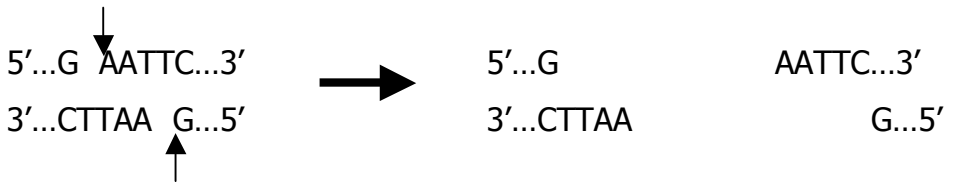
More recently scientists have used these plasmids and biotechnology to produce substances such as human insulin, human growth hormone etc.
Using the bacterium *Agrobacterium tumefaciens* (a plant pathogen), genes can be spliced into plant material. The advent of other advanced instruments and techniques for gene splicing, enables almost all living cells to be potentially transformed with a new piece of DNA. It is finding the gene with the desired expression that is the difficult part and is also the first step in the process of genetic engineering. Extraction and isolation, removal and resplicing of the desired gene will be described further in the following pages.

DNA Extraction:

Two different methods have to be adopted for the extraction of DNA from plant and animal cells. Problems arise with the plant cells in the form of the cell wall. There are two ways this cell wall can be fractured; the first is by using liquid nitrogen in combination with grinding. An alternative method is using an agitating machine that shake the plant tissue in a liquid buffer with a ball bearing. Animal cells don't have a cell wall hence the process can be slightly easier (Table1). A lysis buffer may be all that is required.
Once the plant or animal cell has been opened up and the DNA released, a second problem arises and that is the presence of DNAase enzymes which degrade DNA. These enzymes require certain cations to function properly. By removing these cations such as Mg^{2+} from the reaction by the addition of chelating agents such as EDTA (ethylenediaminetetraacetic acid), the problem is reduced. Addition of detergents such as SDS (sodium dodecyl sulphate) can also inhibit enzymic activity. Other detritus molecules that may cause impurities such as phenolic compounds can be neutralised with PVP (polyvinylpyrollidine). Once extracted and purified the DNA is now ready to be analysed and further research.

Restriction Endonucleases:

Better known as restriction enzymes (R.E.), these occur in bacteria to degrade DNA. These enzymes bind to specific sites on double stranded DNA and cleave it (break it in two). Thus giving the bacterial cells a natural defence against foreign DNA. More than 100 R.E.'s have been identified from different bacteria. All of the R.E.'s have different recognition sites. To see some of these R.E.'s and their recognition sites see the Restriction Enzyme Practical later in this manual. EcoRI (from *E. coli*) is a good example of a restriction enzyme. EcoRI has a recognition site of GAATTC, and the cut is made between the G and A.



Once cut the DNA has been left with an over hang of AATT, this is termed a sticky end. Only other fragments that have been cut with EcoRI can be stuck back into this position. Later you will see how this is used in DNA cloning. Other types of R.E.'s cut straight across the DNA double strand, these are called blunt ends. The pBADgfp plasmid has many R.E. sites; some of these sites are seen in figure 8. Using other R.E.'s (eg HindIII, PstI and EcoRV) and digesting pieces of DNA we can create a restriction map of the pBADgfp DNA. This will show the distance, in base pairs, that the recognition sites are from each other. Each fragment between the cuts can be isolated, sequenced and cloned into a new vector (plasmid). This will give the molecular biologist a chance of finding what genes may be located within that particular DNA sequence (DNA cloning).

Most of the plasmids that have been discovered or created have what is termed the multiple (poly) cloning sites (MCS). This MCS is a location on the plasmid that many R.E.'s cut in close proximity. This site as you we see is also very important in DNA cloning.

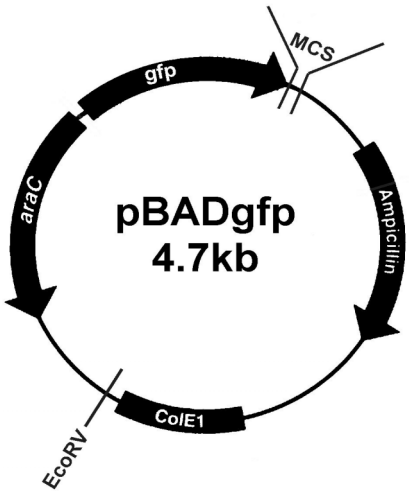


Figure 8: Some approximate positions of the restriction enzyme recognition sites of pBADgfp plasmid. Also termed a restriction map of the plasmid. MCS – multiple cloning site.

Cloning DNA:

The first step in any cloning exercise is the extraction of the DNA required to be cloned, described earlier. If this DNA is originally from eukaryotic cells it is generally digested by the restriction enzyme PstI. The DNA fragments are classified into differing lengths. This can be achieved using gel electrophoresis. All the fragments will feature the same ends

5'……GNNNNNNNNNNNNCTGCA…3'
3'…ACGTCNNNNNNNNNNNNG……5'

NB: N denoting any combination of G, C, T or A. Also that the R.E. PstI cuts with a sticky end.

The plasmid that is to be used as the vector to clone into is similarly digested with PstI. Making sure PstI only cuts once on the plasmid will produce a linear DNA molecule. This linear plasmid will have the same ends as the eukaryotic DNA molecules.
Mixing the plasmid and eukaryotic DNA together in equal molar concentrations will allow them to be joined. The enzyme that is used to join pieces of DNA is

bacteriophage T4 DNA ligase. Further gel electrophoresis can determine whether the new DNA fragment has been cloned into the plasmid. If the base pair size of the plasmid is now larger than the original plasmid size, then the cloning has been successful.

Once the new plasmid has been created, it can be transformed into bacteria. This bacteria can then be analysed to distinguish if it now contains any new traits giving to it be the new DNA sequence inserted.

Polymerase Chain Reaction (PCR)

The PCR method described in 1985 revolutionised the way that molecular biologists analysed their DNA and DNA products. This technique allowed for the amplification of a DNA fragment by approximately a million times. The scientist could accurately increase the quantity of small amounts of DNA for visualisation on a gel. This procedure has now been adopted by, plant breeder looking for specific traits, GM regulators looking for GM contamination of foods, forensic scientists analysing crime scene DNA etc.

The most important discovery for allowing PCR to be achieved, was finding a heat stable DNA polymerase. This DNA polymerase was found in the bacterium, *Thermus aquaticus* and was so named Taq polymerase (shorted to Taq). This enzyme as well as other components such as the dNTP's (ATP, TTP, GTP & CTP) are tolerant to the DNA denaturing temperatures reached in a PCR (Fig 9). PCR is a rapid and highly accurate technique for DNA typing of breeding programmes.

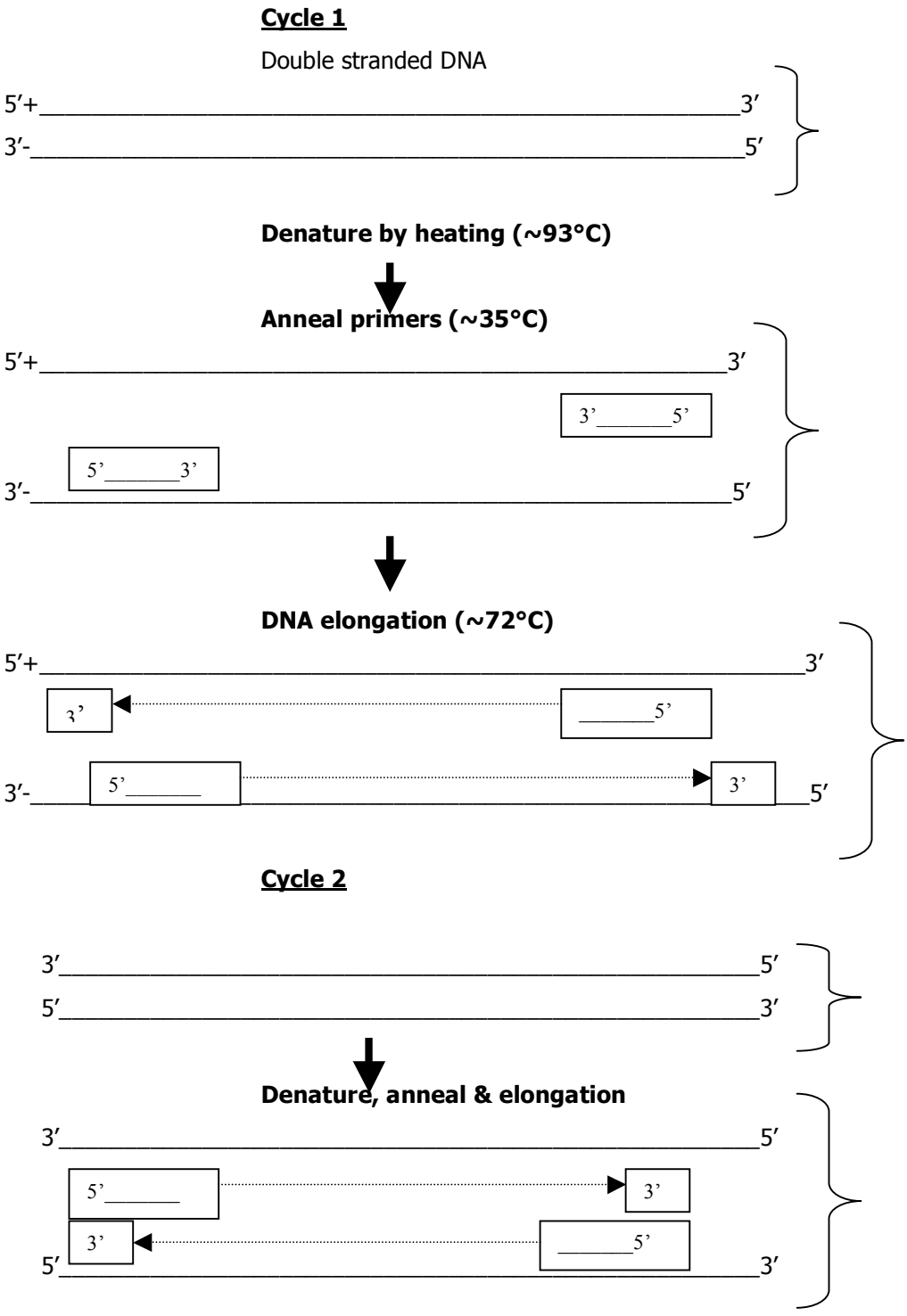


Figure 9: A diagrammatic illustration showing the repetitive cycles of the polymerase chain reaction. The DNA is doubled exponentially with each cycle. After the second cycle each DNA template is the same size.

Fields of Genes
The GM Debate

(Adapted from Landline series, ABC, 2004)

Australia:

1. The group that is concerned about the introduction of GM's into Australia is headed by a person who is a non-GM seed supplier.
   Conflict of interest (Cass)?

2. Biotech companies, e.g. Monsanto and Bayer are pulling money out of Australia because of moratoriums on GM's. Less money for research, so Australia falls behind in intelligential properties, research and knowledge.

3. No moratorium in the state of Queensland due to the cultivation of Bt cotton (*Bacillus thuringiensis*). Cottonseed oil is widely used throughout Australia as a cooking fat, especially in the fast food area like fish and chips. It is so highly refined that it is no longer considered GM, as the oil does not contain DNA. The oil still comes from a GM cultivated plant. Therefore in all states of Australia, consumers are exposed to a GM product, be it cotton or cottonseed oil.

4. A published world map indicating the GM countries in the world shows that the whole of Australia is GM.
   The GM countries on the map are coloured green! Most states of Australia are conducting research trials on GM crops. It seems a little contradictory to have GM products used throughout Australia and we still classify ourselves as a GM free nation (Cass).

5. A new multimillion-dollar research complex built in South Australia for research into GM biotechnology but still there stands a moratorium on GM's in South Australia.
   Why (Cass)?

6. The use of Bt cotton in Queensland has reduced the amount of insecticides applied.
   Maybe a good thing as long as the chemical applications remain low and the insect doesn't build up resistance (Cass).

7. Research trials in most appropriate states are being conducted on the use of Roundup Ready® canola, a GM canola belonging to Monsanto. Would Australia accept a traditionally bred canola? Tradition breeding still includes the cross breeding with other species, chemical, radiation and other mutagenic treatments that alter the genome and produce a new trait.

8. Irreversibility? Once these GM crops are released into Australia can we take them back? Will we stop the gene flow in the natural environment?
   I believe that the irreversibility of genetically manipulated organisms is only as good as the containment we apply. Most genetically modified organisms, being experimental in a research laboratory or grown as a broad acre crop have to be contained. A genetic lab has to be accredited to a high containment rating before any genetic engineering can be undertaken. From a broad acre cropping system, a distinct clear or separation zone of a certain distance must be established around the crop. This clear zone is to prevent (or minimize) any gene flow from the GM crop to the natural ecosystem.
   To describe how well this system works may be best described by using the Canadian canola growers for example. The appropriate GM regulations were practiced and the clear zones established between the canola crop and the surrounding natural ecosystem. Unfortunately a tornado swept across the Canadian plains and spread the GM canola pollen into the natural environment as well as GM free canola crops. Quoting a Canadian canola farmer about this disaster; "The Canadian grown GM free canola is a casualty of war. If you want GM free canola, it may be best to go elsewhere".
   Does this mean that the gene flow of GM canola into the natural ecosystems is irreversible? Probably. (Cass)

9. Can we classify all GM products into the same category?
   Like chemicals some are dangerous but some are also very beneficial. The science of molecular biology is excellent. It may be the application of the science that is questionable! (Cookson)

10. How can we stop herbicide resistance?
    "When you're on a good thing, don't stick to it!" (Powles). By using good rotations in the application of herbicides, the risk of weeds developing resistance will be minimised.
    Typically, when a "fix-all" management strategy is adopted, other beneficial techniques that were used previously are discarded. For example, single dominant gene for 'blackleg' resistance in canola cultivars was released in Australia in 2000. The blackleg disease was not a problem in the crop and many previous management strategies were treated as no longer necessary. This resistance broke down in 2003 and many growers face an uphill battle to reincorporate previous management strategies. Education programmes for growers are important in the future if such problems are to be avoided. (Wherrett)

America:

11. Major GM crops grown in America are corn, soy, cotton and canola. This accounts for 81 million acres of farm land. GM crops were first introduced to the U.S. in 1996. The U.S accounts for 63% of the world's crop. Half of this acreage is allocated to Bt corn.

12. The U.S. has lost markets in Europe, as European countries are not interested in buying GM crops.

13. The Bt gene in a GM crop will not affect ladybirds and butterflies etc. The insect needs to munch on the plant for the Bt toxin to have any effect.

14. Even the Amish population, who shrug modern conveniences, has embraced GM round-up ready soy. 90% of soy grown is GM. This GM soy plant yield no greater than the non-GM, however is better for weed control in the crop. This would eventually lead to an increase in production.

15. Roundup® is a herbicide that knocks down any green plant that is not GM'ed for it or resistant. It is probably hailed as one of the greatest herbicides discovered which is safest to the environment, as it has little residual in the soil. There are specific bacteria that reside in the soil that degrade glyphosate, the active ingredient in Roundup®.
    This is actually where the gene for Roundup® resistance was sourced.

16. With the introduction of crops such as round-up ready soy, the amount of herbicides used was supposed to decrease. Jeffery Smith, 2003 reported in "Seeds of Deception" however that the usage of herbicides was increasing. The pesticide sprays were decreasing.
    Is the increase in herbicide usage a result of more areas being opened up to soybean, corn or cotton growth because weeds that previously couldn't be controlled can now be managed? Or is it an increase per unit area of land? Question the basis of all numbers, on both sides of the argument. (Wherrett)

17. The increase usage of herbicides, mainly Roundup® was great for the company which manufactures this herbicide. That company is Monsanto, one of the biggest producers of GM crops in the world.

18. Monsanto's centre for biotechnology is based in St Louis. Monsanto began its production of GM plants in 1982 when it inserted a gene into a Petunia plant. This centre has 120 controlled temperature rooms, ranging in temperatures that would account for the climates found anywhere from Canada to Western Australia.

19. Monsanto spends US$ 1.3 million/day on research in agriculture. It takes 5-10 years to commercialise a GM crop plant at an estimated cost of US$100 million.

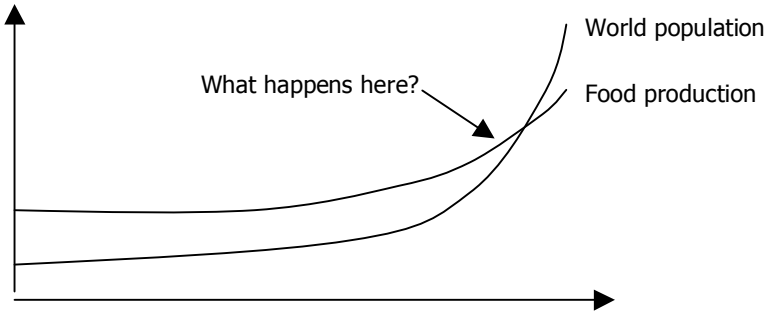
20. Monsanto with all of this investment therefore takes the 'use and reuse' of their seeds very seriously. Growers must enter into a technology agreement when they purchase Monsanto seeds. The grower must not save seed or supply any other grower with seed. One grower in the U.S.A. has been sued and jailed for not adhering to this agreement.

21. Monsanto's herbicide profits are low therefore has to recoup the cost of the GM research from the seed and technology agreements.

22. Bayer does not have technology agreements, but understands why Monsanto does.

23. An estimated 60% or more of foodstuff in U.S. supermarkets are GM. These goods are not labelled as containing GM products.

24. There is a belief in some sectors of the U.S. scientific community that we have to double food production in the next 25 years so has we can feed the world.
This problem was also suggested 150 years ago when the industrial revolution was associated with a period of significant world population growth, but a relative stagnation of food production at the same time. Since that time we have improved irrigation techniques, bred higher yielding crops, introduced fertilizers and pesticides (to name a few). World food production has always managed to keep up with population growth, why shouldn't it now? (Wherrett).



25. Traditional breeding is slow and will not achieve this increase in food production. Genetic engineering is more specific and overall quicker to achieve the sought after traits. Treating DNA like Lego.

26. Americans are more concerned about the fat content of their food compared with the GM content.

27. New GM crops to be developed are; downy mildew resistant roses and gala apples resistant to black spot. Drought and frost resistant, Tastier and more nutritional foodstuff.
Discussion point. An apple rootstock has been genetically modified to give disease resistance to the whole plant. The scion (the bit that is grafted onto the root stock) produces the fruit, but contains no genetically modified DNA. The chemical that induces the disease resistance is created in the root tissue

and transported into the scion. The apples you eat from this tree do not contain GM DNA. What is the problem with that? (Wherrett)

28. Future in Agriculture; Pharming, the cultivation of biopharmaceuticals as a crop e.g. vaccines for pneumonia.

29. Stacked crops; resistant to both herbicides and pesticides.

30. Would Europe, the U.K. and Australia adopt the technology if the GM companies developed the tastier, more nutritional and Pharming crops first?

United Kingdom:

31. Overall belief in Britain is that if humans were in pain, then there would be a willingness to take the risk with GM technology.

32. There is not a lack of food in the world. Even with the developing countries, it is just a matter of distribution of the food.

33. Mankind has fed themselves for 150 000 years with no need for GM's. It is the poverty and trading system that is unfair to the developing countries.

34. The UK may have this attitude, which differs from the US because of recent food scares such as foot and mouth, salmonella and BSE (madcows).

35. Eating beef burgers where shown to be safe after such outbreaks of BSE until 5-6 years later when science discovered this was not true.

36. A lack of trust with the establishment has led people to believe that there is no need for risk.

37. Sceptics are saying that there is not enough testing and research into the human and environmental effects of GM's.

38. Even with the industry pressing the case for GM and the Prime Minister and government in support, until the people's attitude changes, there is no market.

Canada:

39. In the 1970's, the University of Manitoba bred a crop known as canola, adapted and improved from rapeseed. It had improved yields, disease resistance and oil quality.

40. By the 1990's ¾ of canola crop in Canada is GM. With no separation between GM and non-GM at the market place.
    The requirement of separation at the market place creates a logistical problem. Growers have to travel further to deposit seed at a non-GM silo. Do seeds have to be processed at totally different plants? Can the machines ever be cleaned? (Wherrett)

41. 80% of the world canola is bought and sold through Canada, where the world price is set.

42. The GM canola is mostly Roundup Ready®. Growers believe the 10% yield increase is not as important to farmers as is the weed management, which has always been a problem with growing canola.

43. Segregation is one of the biggest issues in Canada, whether it is possible to separate GM and non-GM crops (see also Australia point 8). The president of the Canola Council of Canada states that it is possible to segregate. To set up such a system however is extremely expensive if you want total or low tolerance of separation. Therefore the only way to achieve an economical segregated system is to keep the tolerances at a manageable level. This means, as long as non-GM canola will tolerate a certain percentage of GM contamination, then it is economically manageable!
    Is anything totally segregated? To obtain organic status in Australia no inorganic products can be applied for the 10 previous years. There will always be some residual. So where is the line drawn? (Wherrett)

44. Who pays for the certification of a crop to be GM free? This will undoubtedly be left to the non-GM grower, whose crop may have been contamination by the surrounding GM grower's crop (Cass, 2005).

45. Coordination of planting times may be one way to stop cross pollination of GM and non-GM crops. But what about seed contamination?
    Probably not practical though as sowing times are very tight anyway and yields will suffer. (Wherrett)

46. The National Farmers Union believes that choice is the biggest loser with the introduction of GM technology.

47. Super weeds, a name coined by those not in favours of GM. GM canola crossed with known weeds, which leads to the weeds building up resistances. Herbicide resistance in weeds has been a problem since the 1950's herbicides were first introduced.

48. Bayer who owns Liberty Link® canola (glufosinate resistant), are now concentrating on disease resistance e.g. blackleg resistance.

49. This Liberty Link® canola is a F1 hybrid. Which means that the F2 seeds that are produced by cross pollinating these F1's, may have changed. Therefore there is little benefit in saving seed as other traits such as disease resistance, oil quality and yield will change between seasons. For this reason Bayer does not require a technology agreement.

50. Bayer believes the three most important traits for a new crop that farmers are looking for are; yield, yield and yield.

51. After US$10 million on research into the development of a Roundup® resistant wheat plant, Monsanto has pulled the plant off the market. This they claimed is due to public opinion. However weeds are rarely a problem with wheat crops!

52. A further complication is that Roundup Ready® wheat followed by Roundup Ready® canola will create problems. Canola growing in wheat crops and wheat growing in canola crops and you can't get rid of them!

53. Wheat growers would prefer disease resistance e.g. *Fusarium* resistance.

54. Syngenta seeds have a BT corn that has also got *Fusarium* resistance in the pipeline to hit the market in 2010.

55. It would be a hard task to put the Biotech genie back in the bottle.
Also hard to put the anti-GM genie back in the bottle!? (Wherrett)☺