Recombinant DNA Technology

Dr. Peter Moore BSc PhD

Science Writer, 15 Albert Road, Ashtead, Surrey KT21 1BJ.

The Biochemistry Across the School Curriculum Group (BASC) was set up by the Biochemical Society in 1985. Its membership includes education professionals as well as Society members with an interest in school science education. Its first task has been to produce this series of booklets designed to help teachers of syllabuses which have a high biochemical content.

Other topics covered by this series include: Essential Chemistry for Biochemistry; The Structure and Function of Nucleic Acids; Enzymes and their Role in Biotechnology; Metabolism; Immunology and Photosynthesis.

More information on the work of BASC and these booklets is available from the Education Officer at the Biochemical Society, 59 Portland Place, London W1N 3AJ.

Comments on the content of this booklet will be welcomed by the series editor, Dr. Colin Wynn, School of Biological Sciences, University of Manchester, Oxford Road, Manchester M13 9PT

ISBN 0 90449 830 2

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The Biochemical Society wishes to thank Dr John Lagnado,
Dr Julie Johnston, Dr Michelle Pashley and the NCBE for
their enthusiastic help in the preparation of this booklet.
1. Introduction

Until recently, the complexity of genomes in plant and animal cells has been so vast that detailed examination has been all but impossible. For example, we now know that the human genome consists of some 100,000 genes sited on 23 pairs of chromosomes. Stored away in a code of 3 billion characters are the instructions for every function of our bodies.

However, techniques grouped together under the title of Recombinant DNA technology (Genetic engineering) now allow isolation of small fragments of DNA from cells of any organism, plant or animal. These fragments can then be spliced into a smaller genome, often that of a bacterium. In this new, less complex environment the activity caused by this piece of genetic code can be analysed.

Thus, genes which code for proteins with specific beneficial functions can be isolated and transported to new hosts. Crops can have genes inserted which confer resistance to particular pests; bacteria can be deliberately designed to digest environmental pollutants; sheep can be made to produce proteins in their milk which can be purified into valuable pharmaceutical compounds; diseases caused by a single faulty gene could be cured by giving the patient a new correct copy of the gene - the list is endless.

Applying these techniques has already brought significant changes to industry and medicine. Future applications will transform our lives.

The history of recombinant DNA technology follows a classic pattern for scientific discoveries. At first there was a phase of slow development as a few unconnected theories were formed about fundamental biological processes. Then, quite suddenly, many of these different lines of work converged. There followed a rapid expansion as these theories led to techniques, and the techniques found valuable practical uses.

Once you are at the point of using techniques for practical purposes, more money becomes available, and the rate at which new developments occur increases rapidly.

Many A level syllabuses require students to understand the basics of recombinant DNA technology, asking that they should be prepared to discuss the ethical issues that this new technology raises. However, the ethical implications can only be fully appreciated once students have a working knowledge of the basic principles of this area of science.

For detailed descriptions of the structure and function of nucleic acids, readers are referred to booklet No. 2 in this series.
Basic structure of DNA
We know that the information dictating our physical make-up is stored on chromosomes, made of two chains of a material called deoxyribonucleic acid (DNA).

- The building blocks of DNA, called nucleotides, consist of a phosphate group, a deoxyribose sugar and a nitrogenous base.
- The nucleotides link together via the sugar group, with the 3' site of one joining to the 5' site of another, thus forming long chains. This binding uses phosphodiester bonds.
- There are four types of nitrogenous base in DNA; adenine (A), guanine (G), cytosine (C) and thymine (T).
- Two chains of DNA molecules are held side by side by hydrogen bonds which form between specific complementary pairs of bases. Adenine binds with thymine and cytosine binds with guanine.

**Nucleotide bases**

[Diagram showing the structures of adenine, guanine, cytosine, and thymine, with annotations for their chemical structures and interactions.]
• Whilst the nucleotides within a single strand all face in the same direction, pairs of chains align themselves with their nucleotides facing in opposite directions. The chains are said to be antiparallel.

  5' \rightarrow 3'
  3' \leftarrow 5'

Storing information
• Information is stored in DNA in a code, physically spelt out by the sequence of bases.
• By analogy:
  - bases are the letters;
  - three bases (triplet) form a single word, coding for a single amino acid;
  - a series of triplets forms a sentence (a gene), coding for a polypeptide.
• Each gene is flanked by additional sequences which regulate when it is transcribed.
• The total library of information stored on all of the chromosomes in a cell is called the genome.

Copying DNA
• Double helices separate into single strands.
• A new strand forms alongside each. Because A can only bind to T, and C can only bind to G, this new strand has a sequence of bases that is complementary to the original one.
2. Fundamentals of recombinant DNA technology

Recombinant DNA technology has many applications, all using a few basic concepts. Most procedures exploit molecules and enzymes that are isolated from cells' natural biochemical machinery. Most of our understanding in this area has been derived from work on bacteria and viruses.

2.1 Isolating DNA from cells

When examining an organism's genome, DNA will be extracted from a sample containing many cells, e.g. a blood sample or a piece of tissue. As each cell has a complete set of DNA, the DNA collected will contain many identical sets of code. To analyse the information, the next step is to separate the sections of DNA into groups.

The first stage in working with DNA is to isolate it from the rest of the cell. Methods involve mechanically breaking cells open, and then using detergents and enzymes to degrade cell walls and membranes. The detergents also break down nuclear membranes releasing the DNA.

Cell debris can then be removed by either filtering or centrifuging the extracts. The filtrate will now contain a mixture of DNA and soluble proteins. An enzyme that breaks down protein (a protease) is added. Then, in research laboratories, phenol will be added to destroy all proteins. The DNA can now be precipitated by pouring a layer of ice-cool ethanol over the surface of the filtrate.

Section 4 has a class practical for isolating DNA from onion cells.

2.2 Restriction endonucleases

Restriction endonucleases are enzymes that cut DNA at specific sequences of nucleotides.

In the 1960s Werner Arber and his colleagues found that certain enzymes in bacteria prevented (i.e. restricted) the growth of infecting viruses (phages) by cutting up the viral DNA. It was soon realised that these enzymes always cut the DNA at locations where there were specific sequences of bases. This is now seen as a key breakthrough leading to recombinant DNA technology.
**Blunt ends** - the ends produced by a straight cut  
**Sticky or cohesive ends** - the ends produced by a staggered cut

For example, a restriction enzyme from the bacterium *Proteus vulgaris* called *PvuII* gives a blunt cut at this sequence:

\[
\begin{align*}
5'\text{-C-A-G-C-T-G-3'} & \quad 5'\text{-C-A-G-C-T-G-3'} \\
3'\text{-G-T-C-G-A-C-5'} & \quad 3'\text{-G-T-C-G-A-C-5'}
\end{align*}
\]

Many other restriction enzymes make staggered cuts called sticky ends because of their ability to connect with sections of DNA that have a complementary ending. For example, enzyme *EcoRI* performs the following cut:

\[
\begin{align*}
\end{align*}
\]

Over 500 different restriction enzymes have been identified, each cutting at a specific site and producing a particular type of end. Restriction enzymes are powerful tools for analysing DNA, because they locate specific sequences and cut the DNA in specific ways.

**Naming restriction enzymes**

Restriction enzymes are named after the bacteria they are taken from. For example, the name for all enzymes isolated from *Escherichia coli* strain RY13 start with *EcoR*. This is then followed by a number which simply indicates how many others have been isolated from that strain. So the first enzyme would be called *EcoRI*, the third would be *EcoRIII*. 
2.3 Sorting out the pieces

Having isolated the DNA and cut it into manageable lengths, the next stage is to sort out the various fragments, thus enabling detailed analysis of each.

2.3.1 Gel electrophoresis

*Gel electrophoresis* is used to separate fragments of DNA (or RNA) according to their size. It is commonly used to identify specific cloned genes.

Methods for sorting a sample of DNA into its constituent groups of identical sections make use of two features:

1. Within a sample, fragments of DNA will vary in length.
2. Phosphate groups in DNA give all fragments a net negative charge.

The vast array of fragments produced by the action of restriction enzymes can be separated using a process called gel electrophoresis. The DNA is placed on an agarose gel, and a voltage is applied across it. The charge carried by the DNA will cause it to move across the gel, towards the positive electrode (the anode). Segments of DNA separate because the smaller pieces will move more rapidly than the larger ones, as they are drawn through the sieve-like matrix of the gel. Pieces of identical size will all group in the same place. The rate at which they move is inversely proportional to the logarithm of their length.

Various methods can be used to visualise the DNA fragments once they have been carried into the gel. The simplest involves adding a dye which binds to the DNA and clearly marks the positions of the fragments.

☞ Section 4 has instructions for a class practical.

2.4 Southern blotting

More complex methods of displaying the location of DNA fragments use short synthetic sequences of DNA called gene probes (radiolabelled oligonucleotides). These can be synthesised so that they identify specific
pieces of DNA, and used to mark the presence of specific sequences. Together with the information gained from measuring how fast the fragment moved through the gel, we can discover not only how big each fragment of DNA is, but also start to discover some of the information it is carrying.

Southern blotting DNA fragments are separated by gel electrophoresis. Soaking the gel in alkali denatures fragments into separate strands.

The filter is then removed and treated with radiolabelled oligonucleotides (see page 15) which bind only to specific gene sequences on the denatured strands.

A sheet of X-ray film is laid over the filter and is exposed by the radioactive oligonucleotides. When developed, the X-ray film shows the position of the DNA fragments.
2.5 Amplifying DNA

2.5.1 Polymerase chain reaction

Polymerase chain is a method used to copy fragments of DNA. The two strands of a DNA double helix are separated, and each becomes a template for a new strand. Sequentially repeating the process doubles the number of DNA fragments at each stage.

The technique of polymerase chain reaction (PCR) is extremely powerful and simple to perform. It involves a series of steps, each taking a matter of minutes, that are repeated time and again, so it is easily automated.

PCR makes use of the fact that DNA duplexes separate when heated. A short sequence of nucleotides, a primer, is introduced and becomes fixed to the 3' end of each strand, at the start of the region of DNA we wish to multiply. Single nucleotides and a polymerase enzyme taken from bacteria found in hot springs, and therefore capable of operating at high temperatures, are then added. This causes the primers to be extended, as bases are attached using the original strand as a template. Once finished you have two double strands of DNA. This process can simply be repeated, by again heating the DNA and adding more bases. Each time the cycle repeats the number of fragments doubles, so that after 10 cycles you achieve a thousand-fold increase and after 20 cycles a million-fold increase.

2.5.2 Gene cloning

Having either isolated a specific gene, or created a new piece of DNA *de novo* so that novel proteins can be made, the next stage is to incorporate it into some form of carrying unit, a vector, that will transport it into a host cell. There, the number of copies of the DNA can be multiplied and allowed to be expressed, producing a protein. This process is called gene cloning.
The polymerase chain reaction

1. Double-stranded DNA with known end sequences.

2. Separate chains by heating to 95 °C.

3. Make primer that matches the end sequences of the DNA fragment to be copied. Cool to 40 °C to allow the primer to anneal to each strand.

4. Add heat-tolerant polymerase and bases and heat to 72 °C.

5. Bases add to primers using single strand as a template.

6. Repeat process using the two new double strands.
2.5.3 Vectors used to carry DNA into host cells

There are three basic classes of vector: plasmids, phages and cosmids.

2.5.3.1 plasmids

Plasmids are small lengths of bacterial DNA connected in a continuous loop. They can be modified by adding new sections of DNA, and, as they can easily be placed inside bacteria, they are used to transport foreign DNA into cells.

Plasmid loops usually contain at least one gene for antibiotic resistance, a site where replication always starts, and sites where specific restriction enzymes can make cuts and open the loop. The fragment of DNA to be inserted is prepared so that it has sticky ends which will match those created by the restriction enzyme. Opened plasmids and DNA fragments are mixed together, and join into extended loops as their sticky ends come in contact. The join is made permanent by adding an enzyme called DNA ligase.

Obviously some of the plasmids will simply rejoin, without including the additional DNA fragment. (Many different systems are used to identify bacteria that take up these unmodified plasmids.)

In a process called transformation plasmids are introduced into a bacterial cell. Bacteria and plasmids are mixed in a medium containing calcium chloride, which makes the bacterial cell wall permeable. Plasmids then pass through the cell wall into the cytoplasm. Plasmid uptake is not uniform, and only a small proportion of the bacteria will take up plasmids, though a few will receive more than one copy.

As plasmids contain a gene which confers antibiotic resistance (as well as the gene being tested), adding an antibiotic to the culture medium will kill any bacteria that did not take up a plasmid. This allows direct selection of bacteria that have the modified plasmid.

Once inside the host cell, the bacterial DNA-synthesising machinery is used to replicate the plasmids autonomously. This means that the numbers of copies of cloned DNA can be amplified. In appropriate conditions each cell can produce over 200 copies of the plasmid. Plasmids can easily be modified by inserting a section of DNA containing the sequence you wish to study. Plasmids are limited in that this DNA fragment must be no longer that 10 kb (10 kilobases = 10,000 bases).
Modifying a plasmid

Gene contained in foreign DNA.

Cut foreign DNA and plasmid using the same restriction enzymes.

Site that conveys antibiotic resistance

Origin of replication

Transforming bacteria by inserting plasmids

Bacterium

Plasmid

Chromosome

Place bacteria in calcium chloride.

Cell wall becomes permeable to plasmids.

Plasmid enters bacteria.
Short for bacteriophage, a phage is a virus that specifically infects bacteria. It consists of a linear double helix DNA molecule encapsulated in a protein coat.

T-phages (temperate phages) and λ-phages (lambda phages) are commonly used to carry DNA into *E. coli*.

**Temperate phage**

Lambda phages look similar, except they have no fibres.

Inside the host, the DNA starts to replicate by one of two possible pathways.

**Lytic cycle** - the viral DNA undergoes replication independently of the host chromosome. Vast numbers of viral particles are generated which then burst from the cell and invade other bacteria.

**Lysogenic cycle** - the DNA becomes incorporated into the bacterial chromosome and replicates along with it, slowly increasing in number as the population of bacteria increases.

Phages have the advantage over plasmids in that they can be used to clone larger fragments of DNA: up to 20 kb.
2.5.3.3 cosmids

Scientists now need to introduce DNA fragments that are larger than even phages can carry. To do this a class of vectors called cosmids have been developed. These are hybrids of phages and plasmids.

Produced synthetically, each consists of a plasmid DNA molecule, into which a fragment of DNA of up to 50 kb or greater can be inserted. This is packaged inside a phage. The plasmid is built with sections of the phage’s DNA which are recognised by the phage packaging apparatus. These sections of DNA are called COS sites (COS stands for cohesive). Once built, the phage can introduce this large section of DNA into bacterial cells.
2.6 Cracking the code

2.6.1 Locating specific genes

In order to study the fine structure of normal and abnormal genes, they must be found and then isolated.

2.6.1.1 gene probes

To search for a particular gene hidden away inside a large section of DNA, we must first construct a piece of DNA with a sequence of bases that complements the gene. This will be able to bond (anneal) to the gene, but will not stick to any other part of the DNA.

In 1970 an enzyme called reverse transcriptase (RNA-dependent DNA-polymerase) was isolated from certain RNA tumour viruses. This enzyme builds DNA strands from any messenger RNA (mRNA) molecule. So, first find the mRNA that corresponds to the gene you are searching for.

Some mRNAs are easily purified because in particular tissues they are produced in vast numbers at specific times. For example, the mRNA that encodes silk protein (fibroin) is present in large quantities in the glands of silkworms, and the mRNA molecules that code for milk proteins are numerous in cells in lactating mammary glands.

The routine:
1. Isolate all mRNA.
2. Incubate mRNA with reverse transcriptase in a solution containing adenine, guanine, thymine and cytosine nucleotides as a source of the four bases.
3. The enzyme produces a copy of complementary DNA (cDNA).
4. Detect cDNA using oligonucleotide gene probes.

Reverse transcriptase in action

New molecule of DNA

Free nucleotides

Reverse transcriptase

RNA isolated from cell
2.6.1.2 oligonucleotides

Whilst gene probes are relatively large sections of DNA, oligonucleotides are small, often consisting of no more than 20 bases. Being small, they will only attach to a strand of DNA if there is a perfect match in their sequence; gene probes will attach if the majority of the bases are complementary.

The task is to build a chain consisting of the four different nucleotides arranged in a precise sequence. Oligonucleotides are constructed by deliberately adding nucleotides one at a time, with chain growth occurring in the 3' to 5' direction.

**Building oligonucleotides**

1. The first nucleotide is fixed either to a resin, or to some other large molecule.

2. Single nucleotides are added. They have been modified by fixing a blocking group to their 5' site.

3. The 5' site of the fixed nucleotide binds to the 3' site of the new nucleotide. The clever part is that only one nucleotide can be added, because the 5' site on the new nucleotide is blocked.

4. Excess unbound nucleotides are washed away and then the protective group is removed, exposing the 5' site. Now the molecule is ready to receive an additional nucleotide.

The process can now be repeated, extending the chain by one nucleotide each time.
Oligonucleotides are of great use in looking for mutations within genes, where as little as a single base may have been altered. This is becoming extremely important in genetic screening, as a single base change, resulting in the synthesis of an abnormal protein, may be the root cause of a specific disease.

2.6.2 *Reading the base sequence*

A great deal of information can be gained about a gene’s operation once the sequence of bases is known.

Gene probes are able to locate a particular gene on a chromosome, but they are not able to determine the order of the bases within the entire gene. Restriction mapping, where the genome is cut with a series of different restriction enzymes, starts to reveal small sections of code, as each enzyme cuts only at specific sequences. Digesting a section of DNA with many different restriction enzymes increases the number of known areas. However, as the location at which a restriction enzyme cuts is dictated by a six- or eight-letter code, out of the many thousand bases in a section of DNA, their use can at best reveal the location of only a hundred or so bases.

One possible method is to mark with a radioactive label the end nucleotide on a sequence, clip it off and analyse it. Working one base at a time along a sequence is incredibly time-consuming, and when this was the only method work progressed at a very slow pace. However, with the advent of gene cloning, which provides a way of making large amounts of specific DNA fragments, and gel electrophoresis, which can separate fragments of different lengths, new systems could be devised.

2.6.2.1 *dideoxy sequencing*

Developed by Fred Sanger, this method was used to determine the entire sequence of the 48,513 base pairs in bacteriophage lambda. This was made possible because, like so many aspects of recombinant DNA technology, the process lends itself to automation.

Some of the nucleotides used in the process are radiolabelled, so that the resulting strands can be detected on X-ray film once they have been separated by electrophoresis.
Dideoxy sequencing

Use gene cloning to produce many copies of the DNA fragment to be analysed.

Separate the two chains.

Divide into four samples.

Incubate each sample with a primer in the presence of DNA polymerase and the four nucleotides. New chains grow using the sample DNA as a template.

**KEY STAGE**
Each sample also receives a small amount of one type of nucleotide that has been altered so that it can not form a phosphodiester bond with an incoming nucleotide - these are dideoxy nucleotides. Whilst they can be incorporated into the chain, they block any further growth. A trace amount of a radioactive nucleotide is included to label the growing fragments.

In each sample, chains will stop growing at each of the points where that particular nucleotide is inserted.

Separating fragments by gel electrophoresis produces a series of pieces of known length, each marking the point where the particular base occurs in the sequence. The pattern is revealed by laying an X-ray film over the gel.
2.7 Expression of recombinant DNA molecules

2.7.1 Fundamentals of gene expression
An individual gene supplies the coded information for an individual polypeptide. The process by which this information is transformed is called gene expression. It is divided into two basic parts: transcription and translation.

transcription
First of all the DNA double helix has to separate into individual strands around the start of the gene sequence. This allows RNA polymerase physical access to the chain at the site of a promoter sequence. The enzyme then moves along the DNA molecule starting at the 5' end and building a chain of ribose nucleotides with a sequence that complements the DNA sequence, i.e. the code stored in DNA is transcribed into another code, this time stored in RNA. Once the enzyme reaches the end of the gene sequence, transcription stops and the RNA molecule is complete. In cells with nuclei, this mRNA passes out into the cytoplasm ready for translation.

translation
Now the code can be translated into a polypeptide or protein. Ribosomes pass along the mRNA molecule recognising three bases at a time - a triplet. Each triplet codes for a single amino acid, and the correct amino acid is transferred to the site on the mRNA molecule by specific transfer RNA (tRNA) molecules. Thus the polypeptide chain is built up one amino acid at a time.

2.7.2 Vector design
Taking an isolated gene and simply inserting it into a bacterium is not guaranteed to produce results. Unless the gene is surrounded by appropriate controlling sequences it will remain dormant. This problem is overcome by placing in the vector a fragment of DNA which contains the gene, plus any controlling sequences (promoters) that the host needs in order to recognise and transcribe the gene.

* A detailed description of the mechanism can be found in BASC booklet No. 2 - The Structure and Function of Nucleic Acids.

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However, choosing the exact segment of DNA to make the gene is not always easy as many genes are broken into two or three parts called exons, separated by apparently useless stretches, called introns. When working with these split genes, a decision has to be made whether to insert only the exons or the whole sequence.

Furthermore, vectors can be made to carry genes that have been synthesised in the laboratory. These genes can produce totally novel proteins with unique chemical and physical properties. Cloned genes can also be deliberately mutated to investigate the effect of point mutations on the protein produced, thus helping increase our understanding of some genetic diseases.
3. Applications of recombinant DNA technology and the ethical and safety implications

Recombinant DNA technology has a habit of grabbing headlines, though normally under the more familiar title of genetic engineering. Often the headlines are frightening, predicting out-of-control killer-bugs, monster plants or designer babies. Newspapers are in the business of catching people's eyes and looking for areas of concern, but this approach has the potential for leaving people with an unbalanced impression, as the beneficial uses of technology receive much lower key coverage.

Some of the A and AS level syllabuses ask that pupils should be used to discussing issues surrounding newspaper stories about aspects of genetic engineering. Here are a few pointers to issues that such discussions should try to include:

- What was the initial problem that scientists hoped to solve using recombinant DNA technology?
- How did they set about trying to solve the problem?
- What are the potential benefits?
  a) in the short-term
  b) in the long-term
- What are the potential dangers?
  a) in the short-term
  b) in the long-term
- Is the product of recombinant DNA technology any different from products of conventional industrial techniques? i.e. new varieties of plants can be produced by conventional breeding or recombinant DNA technology
- Does a modified organism harm ecosystems by disturbing a natural balance?
- Could a modified organism lead to economic instability?
- Who is allowed to have access to the information, i.e. patenting genes; genetic screening and insurance companies?
- Where appropriate, is a particular application so important that it justifies using animals as part of the process?

In this section we will review some of the current applications of recombinant DNA, looking at the advantages and potential pitfalls.
3.1 Health care

Because health care is so important, it should come as no surprise that almost every new technology rapidly finds an application. Recombinant DNA technology lends itself to the world of medicine in a number of different ways.

3.1.1 The Human Genome Project

The Human Genome Project is a highly co-ordinated international project that intends to map the entire human genome.

Co-ordinated by the Human Genome Organisation (HUGO), this multi-billion pound project aims to produce a map of the entire genome on the 46 human chromosomes. The genome has been divided into a number of different regions and some 250 individual laboratories around the world are working on sequencing each part.

The potential benefit for medical research is immense, as it will accelerate the rate at which genes can be located. Once the genes are located, genetic screening for many more conditions will be possible. In addition, it will increase our understanding of the codes which surround genes and control their activity.

However, this is not without its difficulties. Once located, a gene suddenly becomes valuable. Research companies have been quick to apply for patents over sequences, so that they can recoup the money they spent finding it. At one point, more than 5,000 patent applications were made for sections of code without even knowing their functions. After an agreement between authorities in the UK and the US these applications have been withdrawn. But this raises an ethical issue - as the companies did not design the gene, but only found it, do they have the right to patent it?

So, whilst patents for genetically altered micro-organisms are now routinely granted by the US, European and Japanese patent offices, patents covering human genes are still rare.
3.1.2 Diagnosis of genetic disease

**Diagnosis of genetic diseases**: looking for gene mutations or deletions that cause specific diseases.

In taking genetic material from a few cells and rapidly multiplying it, this technology has opened many doors for detecting disease. White blood cells from a small blood sample, a few cells scraped from the inside of a person’s mouth, or even cells from a fetus that are floating in the amniotic fluid, can supply all the information needed for complex genetic diagnosis.

At the moment, diagnosis is restricted either to conditions that result from a person having an incorrect number of chromosomes (such as Down’s syndrome, where the extra copy of chromosome number 21 can be detected), or to conditions caused by a defect in a single gene. However, of the 4,344 recorded diseases caused by single gene mutations, many are extremely rare, and as yet very few gene probes have been built that can identify them. For the more common diseases, such as cystic fibrosis or haemophilia, gene probes have been built. Such probes can be used either to see whether an individual is carrying a faulty gene and may therefore pass it on to any offspring, or to analyse the cells of a developing embryo and see if the faulty genes have been passed on.

The occurrence in a population of a particular disease caused by a recessive gene can be calculated once you know how many adults carry a single copy of that gene. The calculation assumes that there are no social circumstances that may influence the pairing of a couple, and the fact that recessive diseases will only be passed to a couple’s offspring if both parents carry the faulty gene. For example, one in every 25 adults carries a single copy of the gene which causes cystic fibrosis. However, the number of people who have cystic fibrosis is less than one in every 2,500.

- If one in 25 adults carries a single copy of the faulty gene, the chance of both partners carrying the same faulty gene is 1 in 25 x 25 .................. 1 in 625
- If both parents do carry the faulty gene, the chance of their offspring receiving two disease-causing genes is .............. 1 in 4

**: The occurrence within the population of the disease will be ..... 1 in 2,500**
Great care is needed when advising couples about the likelihood of their giving birth to an affected child. Such complex and difficult advice is part of the job of genetic counsellors.

**genetic screening**

Taking the ability to look for genetic disease one step further, brings us to genetic screening. This gives people the capability to find out not only that their children may be at risk of some disease, but that they themselves may develop some particular disease later in life. The consequences of such information are enormous. A person may be able to avoid certain jobs, because they now know that owing to their personal genetic make-up, the job would be harmful. On the other hand, the information may be used by employers to refuse employment to certain individuals.

Another problem is that insurance companies may ask for higher premiums from individuals whose genetic tests show that they are more susceptible to a particular disease. Alternatively, anyone who can show that their risk is low may get cheap insurance.

### 3.1.3 Gene therapy

*Gene therapy*: treating a genetic disease by inserting a healthy copy of a gene into the cells that particularly need it.

*Somatic therapy* treats cells of the body which are not involved in reproduction; any alteration will not be carried to future generations.

*Germ-line therapy* treats cells that make eggs and sperm, therefore affecting all future offspring.

In the spring of 1993, Carly Todd became the first patient in the UK to be treated with gene therapy. The treatment was also the most advanced of any performed. She had a single gene disease called adenosine deaminase (ADA) deficiency. The cells in her body could not produce working copies of the enzyme ADA because of a fault in the gene sequence. Whilst all cells use ADA, the white blood cells are particularly vulnerable if it is absent. Consequently, Carly had almost no white blood cells and, as white blood cells form a vital link in our immune systems, she could not fight off infections (a condition called severe combined immuno deficiency).
White blood cells are manufactured by bone marrow cells. If a correct version of the gene could be put into her bone marrow cells then they would be able to make healthy white blood cells. In a simple operation, doctors collected a sample of her bone marrow. They had previously isolated and cloned the gene for ADA, and packaged it in a viral vector. Mixing the bone marrow cells and the modified viruses in a laboratory allowed the gene to be passed into some of the cells. These cells were then returned to Carly. The hope is that over the next few years these cells will multiply, populate her bone marrow and produce sufficient healthy white blood cells to form a natural defence against infections. It will be a couple of years before we know whether the treatment has been a success. In the meantime her health is protected with a variety of drugs.

The future holds many possibilities for gene therapy, but there are some major restrictions to its implementation.

isolated and cloned
The gene responsible for the disease must be known, isolated and cloned. This is still the case for only a few conditions. Before inserting the gene it must also be surrounded by correct controlling regions. Without these it could either fail to cause any protein production, which would be a waste of time, or, it could cause an over-production of the protein, which may be damaging.

location of effect
Gene therapy will only work if the gene can be placed into the cells that particularly need to use it. Again this narrows the field, as the process by which a faulty enzyme causes a disease, and hence the exact cells which need treating, is known in a remarkably small group of genetic diseases. Having discovered which cells need targeting, you still have to get the gene into them. In the case of ADA deficiency this was done by removing the appropriate cells from the patient, treating them outside her body and then returning them.

However, this will not always be possible. For example, cystic fibrosis affects cells throughout the body that are involved in producing secretions. An affective gene therapy will therefore need to get the gene to all of these cells. For treating the respiratory problems associated with cystic fibrosis, the gene can be placed in an aerosol that can be inhaled in much the way that asthma drugs are inhaled, but treating secreting cells not on the body's surface may prove more difficult.
single gene defects
If treating conditions resulting from only a single faulty gene is so hard, it is
difficult to see how the techniques could be applied to conditions that result
from a defect in more than one gene.

somatic versus germ-line therapies
Whilst treating a disease using gene therapy can be considered to be an
extension of conventional medicine, altering the genetic make-up of sperm
and ova has much wider implications. At the moment germ-line therapy is
banned in the UK even though it holds the possibility of eradicating diseases
(e.g. haemophilia) from future generations. What are the problems?
Once a gene has been inserted into the germ line, it has the potential to
be inherited by all future generations. The consequences of this are
unknown. There is not yet enough information about what happens to genes
once they have been placed in cells and whether they affect the functions of
other genes.

3.1.4 Transgenic organisms

Transgenic organisms: an organism that has had its
genetic make-up altered by transferring into it a gene from
another species. As a result it manufactures a protein that it
would not normally produce.

One of the greatest areas of potential commercialisation, and not
surprisingly of controversy, is the use of recombinant DNA technology to
move a gene deliberately from one organism to another. This procedure is
often carried out to produce commercially valuable proteins like hormones
or drugs.

micro-organisms
As we have seen, once a gene has been isolated it is relatively easy to move
it into a bacterium. Once in place, the bacterium can manufacture the
protein coded by the gene. Placing the human insulin gene in bacteria has
been successfully used commercially to produce human insulin, a vital drug
for people with diabetes. Another example is the yeast cells that have had
DNA incorporated so that they manufacture a hepatitis B vaccine.
animals

If a gene is inserted into the nucleus of a fertilised egg cell before it starts to divide then every subsequent cell will contain the new gene. This technique has been used to place a gene that codes for alpha-1 antitrypsin into a sheep embryo. The gene was surrounded by controlling sequences that allow it to be expressed only in mammary glands. As a result, this transgenic sheep produces milk that contains this protein. Alpha-1 antitrypsin is used to treat patients with a particular type of inherited lung disease - congenital emphysema.

More radically, transgenic animals may become a valuable source of transplant organs. When patients receive transplanted organs, one of the biggest problems they face is that their bodies’ immune systems recognise that proteins on the surface of cells are foreign - they come from a different individual. The immune systems then set about destroying the new organ. To prevent this process of rejection, transplant patients are given drugs that suppress their immune systems. However, this then leaves them vulnerable to disease. Using recombinant DNA technology, it may be possible to transfer to a developing pig embryo, the parts of a person’s genetic sequence that produce proteins which allow recognition of their own organs. When transplanted, the pig’s organs would then carry proteins that would fool the patient’s immune system into thinking that the organ was not foreign. The organ would then not be rejected and no immunosuppressants would be needed.
3.2 Agriculture

Never before has such a demand been placed on the world’s agriculture. Today the global population exceeds 5 billion, and is expanding by 84 million people each year. By the turn of the century it is expected to reach 6 billion. Recombinant DNA technology is being applied to increase yields of both plants and animals, to increase resistance to disease or pollution and to create new crops that can utilise previously wasted resources.

A popular method for introducing DNA into plants uses a strain of bacteria found in soil (*Agrobacterium tumefaciens*). In their natural state the bacteria infect plant cells, inserting part of their DNA and causing cancer-like growths. New genes can be inserted into a section of the bacterium’s DNA, to transfer the genes to plant cells. As tobacco plants are particularly susceptible to infection by this bacteria, much of the fundamental research has been carried out on them, with the intention of using the knowledge gained to help develop food crops.

Recombinant DNA has yet to make great in-roads into the genetic code of many farm animals, although experimentally, growth hormone genes have been inserted into embryos, along with genes that affect meat quality. However, many production-enhancing drugs or specialised animal feeds are being manufactured using microbes that have been altered by recombinant DNA technology.

3.2.1 Improving yields

There are different ways of increasing yields. You could either aim for a faster rate of growth, for more efficient use of nutrients, or for an increase in the quantity of the food produced by each organism.

In the case of cereal crops like wheat or rice, much attention has been given to increasing the number of grains that grow in each head, whilst causing the plant to grow with a shorter stem, thus wasting less energy on this inedible part of the plant. In the past, such development was carried out in prolonged breeding programmes, but now the rate of development can be accelerated.

Recombinant DNA technology has also been used to manufacture bovine somatotrophin (BST), a hormone that increases by 10 to 15 per cent the amount of milk that a cow can produce. The gene has been placed inside bacteria which makes them produce the hormone. However, this example highlights some of the potential problems of implementing the technology. In Europe, there is a surplus of milk. Making cows produce more milk
means we will need less of them. Consequently some farms will no longer be needed. Also there are fears that the hormones harm cattle, leading to an increase in the numbers that suffer from infections of the glands that make milk in their udders. Furthermore, milk from cows injected with BST have higher levels of a growth-controlling protein (insulin-like growth factor-1), and no one knows what effect that may have on people who consume it. At the moment BST is banned in Europe, but allowed in the US.

**nitrogen fixation**

A major area of research is dedicated to finding ways of moving nitrogen-fixing genes (NIF genes) into agricultural crops. Nitrates are vital nutrients for most plants, and some bacteria are particularly good at creating them by biochemically reducing nitrogen. Plants of the legume family (e.g. peas, clover, lucerne) foster these bacteria in highly specialised root nodules. Using energy stores from the plant, the bacteria fuel nitrogenase enzymes to reduce atmospheric nitrogen to ammonia, which is then taken up by the plant and converted into a number of nitrogenous compounds. When the plant dies these are left in the soil. If cereal crops are grown on this enriched soil in a following year the extra nitrates lead to a better yield. Alternatively, farmers spread expensive nitrate fertilisers on their land to boost their crops.

However, all this might change. The genes which produce the necessary NIF enzymes have been isolated, sequenced and cloned in *E. coli*. Now scientists are looking for ways of placing them into cells in crops such as wheat or rice. This would enable these crops effectively to fertilise themselves, saving much money and increasing yields. Placing the gene in crops grown in developing countries would have enormous impact on their ability to grow food, as currently they can not afford the nitrogenous fertilisers used in more affluent countries.

**3.2.2 Resistance to disease, pests or herbicides**

Many crops are particularly vulnerable to attack from specific organisms, e.g. tomatoes are attacked by aphids, potatoes by viruses, wheat by fungi. Farmers fight back by spraying their crops with chemicals that destroy the invading organisms.

Now recombinant DNA technology is allowing plant breeders to introduce genes into crops, causing them to produce their own pesticide. For example, a tomato plant has had a gene placed in it that produces a protein which kills tomato fruit worms. When modified tomato plants and normal plants were grown together in a laboratory and deliberately infected with
fruit worms and pin worms, the normal plants were stripped of their leaves, but the modified plants were unaffected.

**benefits**
- increased resistance to attack
- increased yield because crop damage is reduced
- in some cases there will be no need to spray fields with chemicals that are costly and potentially dangerous

**risks**
- such genes may get into weeds, giving them resistance to attack and allowing them to flourish
- when the crop is eaten, you would also consume the genetically produced chemical, which may be harmful
- the attacking organisms may become resistant to the chemical and so the process gives no protection

In a similar way crops can be adapted to make them resistant to herbicides. Most herbicides operate by blocking an enzyme that is vital for the plant's survival. The idea is to equip plants with a gene which produces an enzyme that can destroy the herbicide. When the field is sprayed with the specific herbicide, the genetically modified plants will be unscathed, whilst all other plants will be killed.

In June 1994, the first transgenic plant was licensed for agricultural use in Europe. It is a tobacco plant that has built-in resistance to the herbicide bromoxynil.

**3.2.3 Novel crops**

One of the problems of modern agriculture is that a crop is frequently grown many hundreds or thousands of miles from the customer. It can be difficult to get it to shops before it rots. Fruit and vegetables are often harvested before they are ripe and then ripened artificially just before they are sold. This leads to a loss of flavour.

In the case of tomatoes the problem may now have been solved - researchers have now produced a tomato that is resistant to becoming soft and rotting once it has ripened. Fruit normally softens because it produces an enzyme called polygalacturonase (PG). This enzyme breaks down pectin in cell walls and so the fruit becomes soft. By inserting a gene which has a sequence that is exactly the opposite of the gene coding for PG (an anti-
sense gene) the cells produce an anti-sense strand of mRNA as well as a sense strand from the normal gene. These two mRNA strands are so perfectly matched that they stick together, thus rendering the sense strand useless. Without the mRNA no enzyme forms, and without the enzyme the fruit stays firm.

How an anti-sense gene works

1. Sense strand in the normal DNA (the gene).

2. Produces a sense strand of mRNA.

3. Anti-sense strand of DNA introduced into cells.

4. Produces an anti-sense strand of mRNA.

5. Anti-sense strand binds to sense strand and blocks its activity.

Some people are allergic to some of the proteins found in milk. In the same way that sheep can have genes inserted causing them to produce valuable pharmaceutical proteins in their milk, they could have genes inserted that modify the milk, removing these allergenic proteins. In the future there could be many new niche markets for supplying tailor-made foods.
3.3 Food and drink

We have already seen how recombinant DNA technology is affecting food production in standard agricultural situations. Now we turn to the other areas of the food industry that are making use of the techniques.

3.3.1 Microbial protein

Bacteria can be designed to grow on virtually any energy-rich molecules. Some have been adapted to use methane gas as a nutrient and others grow successfully on paper pulp. This is already providing a new source of protein to the food industry. The growing bacteria can be harvested and their proteins purified. These proteins may be of particular value as the numbers of people eating a vegetarian diet increase.

benefits

- Growing farm animals to supply us with protein is a very inefficient use of energy as it can take between 10 and 20 kg of protein in feeds to produce 1 kg of meat protein. However, bacteria are much more efficient.
- Waste materials such as pulped newspapers could form the basic nutrient supply for new bacteria.

risks

- Care will need to be taken that the bacteria do not contain proteins to which people are allergic.
- If a microbe is designed to digest cellulose efficiently, care will be needed in the way it is contained. Were viable cellulose-digesting bacteria to escape into the environment, they could devastate anything made of paper or wood.

3.3.2 Microbes in food production

Industrial cheese production uses a lot of an enzyme called chymosin (commonly called rennin) to coagulate the protein casein, found in milk. Traditionally this enzyme is obtained from the contents of the abomasum (fourth stomach) of suckling calves when they are slaughtered for veal. However, the numbers of calves being slaughtered is decreasing, but the quantity of chymosin required for cheese making is increasing.
Bacteria are modified by transforming them with the inclusion of a gene that causes chymosin production. There are seven basic steps.

1. mRNA for all of the sequences needed to make chymosin is isolated from calf stomach cells.
2. This m-RNA is transcribed into cDNA.
3. cDNA is cloned into a vector.
4. *E. coli* are transformed by the vector (*Kluyveromyces lactis* and *Aspergillus niger* are also used).
5. Standard fermentation techniques are used to grow the *E. coli*.
6. *E. coli* are harvested, broken down and the enzyme purified.
7. Tests show that no bacteria are present in the extracted enzyme, and feeding trials with rats showed that they were not harmed by consuming a dose of the enzyme that was 100,000-fold greater than expected for the average human consumption. It is therefore safe to use in cheese making.

Such bacterial chymosin is used in the production of vegetarian cheese.

### 3.3.3 Detection of food-borne pathogens and spoilage agents

Recombinant DNA technology has also come to the help of food manufacturers who need to know whether a product is safe to eat, or whether it is contaminated with pathogenic bacteria. A series of gene probes have been built that carry sequences which can specifically identify the presence of a wide variety of different food pathogens.

Some bacteria, such as *Listeria monocytogenes*, are only dangerous if they are alive. Whilst conventional tests take one to three days to give results, gene probes can do the job in a few hours. Gene probes for sections of DNA in *L. monocytogenes* have been built, and these can be used to see whether the bacteria are present. But as DNA lasts for thousands of years once the bacteria are dead, this will not distinguish between dead and live organisms. However, a new variety of probes has been built, this time coding for the mRNA sequence. As mRNA is only present in living cells this will only detect bacteria that are alive.

### 3.3.4 Labelling genetically modified foods

Owing to public concern about the use of recombinant DNA technology to move genes between plants, some people think that genetically modified foods should be labelled. This would also include any foods that have been manufactured using genetically modified crops as ingredients.

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foods are likely to be labelled if they have been modified by inserting:

- a copy of a human gene
- a copy of a gene taken from an animal which is the subject of religious dietary restrictions, e.g. a gene from a pig

Also, to help vegetarians decide which foods to eat, crops that have been modified by adding copies of genes that originally came from animals will probably have to be labelled.

problems behind labelling:

- it will be very difficult to police because many genetically modified plants and animals will be almost identical to varieties produced by conventional breeding programmes
- it could cause unnecessary anxiety as people may think that the labels are warning that the food is unsafe
- how much information can you fit on a food packet?
- will people understand the information you are giving them?

3.4 Environment

3.4.1 Releasing genetically modified organisms

Releasing genetically modified plants, animals or microbes into the environment is regulated by law and a licence has to be obtained for each product before any release is allowed. There are few risks associated with the process of adding a new gene to an organism, but there are risks that result from designing organisms that have new combinations of genes.

However, it is not always easy to identify what is meant by ‘releasing’ a genetically modified plant or animal into the environment. For example, a genetically engineered sheep in a field might not be considered to have been released, so long as the fences are strong enough, but a genetically modified bacterium placed in the same field would be considered to have been released. The key to the argument is whether you can contain the organism. Whilst a sheep is contained by fences, bacteria need much more stringent physical barriers. Basically you have to ask ‘can it escape?’
**knock-on effect**

Recombinant DNA technology can be used to alter bacteria or small animals, such as beetles or worms, so that they selectively destroy particular pests (e.g. a nematode has been altered so that it selectively destroys snails and slugs). This will save using agricultural pesticides which are harmful and expensive. However, by introducing a new predator into an ecosystem we deliberately alter its balance. It is impossible to predict fully the outcome of such a change. Such an alteration may not necessarily be harmful, but it needs to be monitored carefully each time a new organism is released.

The danger is that if any particular organism does cause harmful knock-on effects, there may be great problems removing it from the environment. Consequently, the law stipulates that trials are first of all conducted in a laboratory, then in a small experimental plot, then in a contained area of a field, before finally allowing full release.

Some fish have been genetically altered so that they have an extra growth hormone gene. They also have an additional gene making them produce a type of anti-freeze in their blood. This enables them to survive in very cold water. If they were released, either deliberately or accidentally, these fish might initially supply an extra source of food. As time goes on they may disturb the native populations of fish and deplete food reserves.

**self control**

At the same time as adapting an organism so that it performs a particular function, it is possible to adapt it so that its ability to survive is limited. This can control its spread once released.

- Genes can be added that cause an organism to require particular nutrients that are not normally found. When you deliberately stop supplying this nutrient, the organism dies.
- A suicide gene could be inserted. For instance, a bacterium could be designed to destroy a particular pollutant, such as crude oil, and a gene could be inserted that kills the bacterium if there is no crude oil around. Therefore the bacteria can be sprayed onto an oil-spill, they will destroy the oil, and when all of the oil is gone they will kill themselves.

**3.4.2 Economic considerations**

Many crops can only be grown in particular countries, because those countries have hot climates. The ability to move genes into tropical plants that will then allow them to be grown in temperate regions, may at first sight seem a good idea. However, such crops would then destroy the economies of many tropical countries. For instance, many Caribbean
countries depend on their sales of bananas for foreign income. If bananas could be grown in the UK then we would have no need to go to the expense of importing them. This would severely damage the economies of the exporting nations.

On the other hand, recombinant DNA technology could be used deliberately to design new crops to grow in arid conditions. These could be grown by farmers in areas of the world which suffer from food shortages, transforming their standard of living. The problem here is that such crops are costly to develop and poor countries can seldom afford to buy them.

3.4.3 Handling pollution

As we have seen, microbes can be designed to grow on many waste materials to produce useful materials such as food. They can also be usefully employed to control pollution.

- Bacteria have been designed to break-up oil slicks.
- Bacteria have been designed to destroy noxious gases released from factories. Fumes are pumped though pipes running under gravel or wood chippings, which supply a large surface area on which the bacteria can grow. As the gases leave the pipes they pass through the filter-bed and the bacteria ingest and destroy the noxious components.
- Plants and animals can be designed so that they will grow in polluted environments. However, this may have a negative effect on controlling pollution, as it could discourage people from spending time and money cleaning-up the environment and allowing the native population of plants and animals to live there.

3.5 Forensic science

When investigating a criminal offence, such as a burglary, serious sexual assault or murder, the police are trying to find evidence that will indicate who the criminals are. Recombinant DNA technology has now been used in a number of different ways.

3.5.1 DNA fingerprinting

The best known example is DNA fingerprinting, which is based on two assumptions:
1) Each of a person’s cells carries an identical set of DNA
2) The DNA code for every individual person is unique
This means that if a few drops of blood or the few hair follicle cells attached to pieces of hair (or semen in the case of a sexual assault) are left at the scene of the crime, then the DNA can be extracted, multiplied and then analysed. The results can then be matched to those of a similar analysis carried out on samples taken from a suspect. If they match, this indicates that the suspect was at the crime.

What unique features does the test look for?
The sequences which code for genes are very similar throughout the population (they are highly conserved). However, large stretches of DNA contain short sequences between 15 and 100 nucleotides long that repeat over and over again. The numbers of repeats in these areas of DNA are very different between individual people. Oligonucleotide probes which code for one of the repeating sequences are used, each person’s DNA producing a unique pattern.

**A genetic fingerprint**

DNA is extracted from sample, cut using a restriction enzyme and the fragments are separated by gel electrophoresis.

DNA fragments are transferred from the gel onto a sheet of nylon.

Nylon sheet is soaked in a bath containing oligonucleotide probes which code for frequently repeating sequences in DNA.

As the oligonucleotides are labelled with a radioactive molecule, their position can be detected by laying a sheet of X-ray film over them.

Bath containing oligonucleotide probes

Distinctive DNA fingerprint
4. Practical work

There is a variety of ways that recombinant DNA technology can be demonstrated in a classroom, either using practical experiments or simulations. A few ideas are listed below.

DNA origami kit
This 'Whodunit?' kit is designed to show students how a number of commonly used molecular biological techniques are combined to produce a DNA fingerprint. The steps in this simulation all have an equivalent in the real process.

Each student is provided with paper strips marked with 'restriction sites'. After cutting the strips they are 'electrophoresed' by aligning them on a board. Applying a solution develops the strips and reveals a unique series of bands.

For details contact Dr. J. Johnston, Royal Holloway College, University of London, Egham, Surrey TW20 0EX (Tel. 01784 4437563).

Lambda phage on a disk
The entire sequence for the bases in lambda phage has been found. It can be obtained on a computer disk in a form that can be read into standard word processors. Using the search/find functions, restriction sequences can be found and the bases divided into fragments. The count facility will rapidly tell you how many bases there are in each fragment.

For details contact: Science and Plants for Schools, Homerton College, Cambridge CB2 2PH (Tel. 01223 411141 ext. 233).

Practical experiments
The National Centre for Biotechnology Education has designed a series of practicals that can be performed in school laboratories with minimum investment. They make use of simple equipment and heat stable enzymes. Two work sheets are reproduced on the following pages with permission.

For details contact: National Centre for Biotechnology Education, Department of Microbiology, University of Reading, Reading RG6 2AJ (Tel. 01734 873743).

Courses
Both the NCBIE and SAPS run courses to train science teachers - contact them for details.
4.1 The lambda protocol - electrophoresis

1. Flick to mix

2. 100 µl Distilled water
   2 µl Dried λ DNA

5. Molten agarose 55°C
   Tank
   Comb

6. Loading dye
   2 µl
   IMPORTANT! Mix contents of tubes thoroughly
   Buffer solution
   Black card
   Carbon fibre tissue
3. Lamb DNA solution

- 20 µl of EcoRI
- 20 µl of HindIII
- 20 µl of BamHI
- Uncut DNA ('Control')

4. Incubate for 30-45 min

7. Carbon fibre electrodes

8. Wells DNA bands

- Loading dye
- DNA stain

WARNING! < 25 volt
DNA from onions

Add 2-3 drops of protease enzyme to about 6 ml of the washing-up liquid / DNA mixture.

Very carefully trickle about 9 ml of ICE COLD alcohol onto the surface of the washing-up liquid / DNA mixture.

The DNA precipitates into the (upper) alcohol layer.

DNA

Add the salty washing-up liquid solution to all the chopped onion.

Incubate the mixture at 60°C for exactly 15 minutes.

Strain the mixture through a coffee filter.

Chill the mixture by standing it in a jug of ice for a few minutes.

Blend the chilled mixture for no more than 5 seconds.

10 ml washing-up liquid
3 g. salt
100 ml water
5. **Helpful Books**

**An Introduction to Genetic Analysis**  
Griffiths, Miller, Suzuki, Lewontin and Gelbart  
WH Freeman and Company  

**Biotechnology**  
J.E. Smith  
Studies in Biology No. 136  
Edward Arnold  
ISBN 7131 29603

**Biotechnology (Extending Science Series No. 11)**  
J. Teasdale  
S. Thornes Ltd  
ISBN 0859 50555 3

**DNA Science - A First Course in Recombinant DNA Technology**  
D.A. Micklos and G.A Freyer  
Cold Spring Harbour Laboratory Press  

**Microorganisms**  
J.I. Williams and M. Shaw  
Collins Educational/Bell & Hyman  
ISBN 000 322324 8/7135 1321 7

**The Structure and Function of Nucleic Acids**  
C.F.A Bryce  
The Biochemical Society  
(Tel. 0171 580 5530)

*For practical classes*

**Practical Biotechnology - A Guide for Schools and Colleges**  
National Centre for Biotechnology Education  
Department of Microbiology, University of Reading (01734 873743)

**Revised Nuffield Advanced Biology**  
Study Guides, Teachers’ Guides and Practical Guides  
Longman  
ISBN 0582 354315

*Videos*  
**The Biochemical Basis of Biology**  
Video No.3: Manipulating DNA  
E.J. Wood  
Portland Press Ltd (Tel 01206 796351)
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