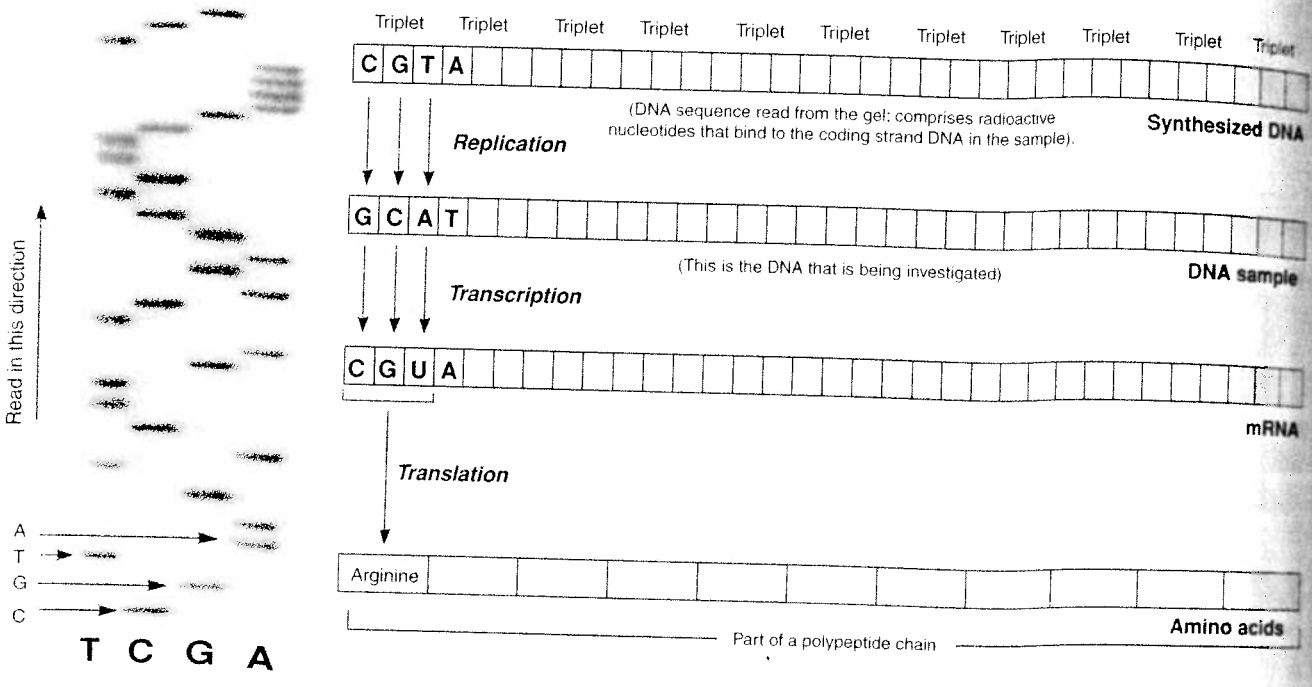
Analyzing a DNA Sample

The nucleotide (base sequence) of a section of DNA can be determined using DNA sequencing techniques The base sequence determines the amino acid sequence of the resultant protein therefore the DNA tells us what type of protein that gene encodes. This exercise reviews the areas of DNA replication, transcription, and translation using an analysis of a gel electrophoresis column. **Attempt it after you have completed the rest of this topic.** Remember that the gel pattern represents the sequence in the synthesized strand.

1. Determine the amino acid sequence of a protein from the nucleotide sequence of its DNA, with the following steps:
   (a) Determine the sequence of **synthesized DNA** in the gel
   (b) Convert it to the complementary sequence of the **sample DNA**
   (c) Complete the **mRNA** sequence
   (d) Determine the **amino acid** sequence by using the 'mRNA amino acid table' in this workbook.

   NOTE: The nucleotides in the gel are read from bottom to top and the sequence is written in the spaces provided from left to right (the first 4 have been done for you).



2. For each single strand DNA sequence below, write the base sequence for the **complementary DNA** strand:

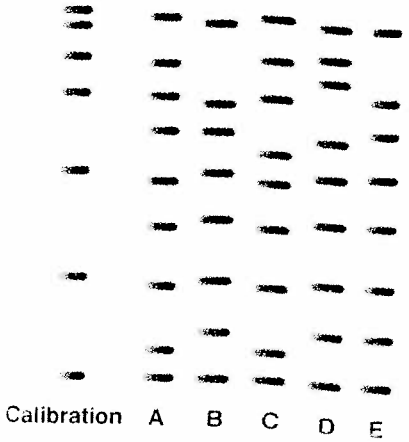
   (a) DNA: T A C T A G C C G C G A T T T A C A A T T

   DNA: _____

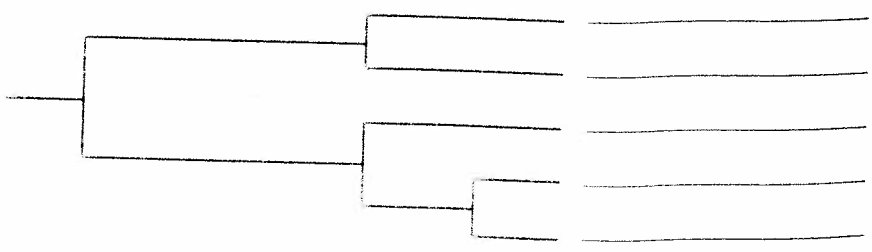
   (b) DNA: T A C G C C T T A A A G G G C C G A A T C

   DNA: _____

   (c) Identify the cell process that this exercise represents: _____



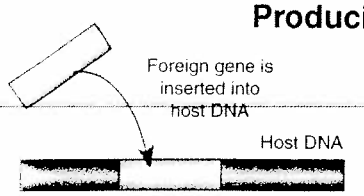
3. Determine the relatedness of each individual (A-E) using each banding pattern on the set of DNA profiles (left). When you have done this, complete the dendrogram by adding the letter of each individual.



What is Genetic Engineering?

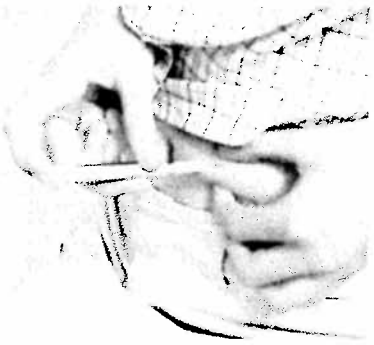
The genetic modification of organisms is a vast industry, and the applications of the technology are exciting and far reaching. It brings new hope for medical cures, promises to increase yields in agriculture, and has the potential to help solve the world's pollution and resource crises. Organisms with artificially altered DNA are referred to as **genetically modified organisms** or **GMOs**. They may be modified in one of three ways (outlined below). Some of the current and proposed applications of gene technology raise complex ethical and safety issues. The benefits of their use must be carefully weighed against the risks to human health, as well as the health and well-being of other organisms and the environment as a whole.

Producing Genetically Modified Organisms (GMOs)

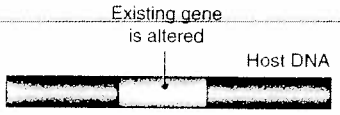


Add a foreign gene

A novel (foreign) gene is inserted from another species. This will enable the GMO to express the trait coded by the new gene. Organisms genetically altered in this way are referred to as **transgenic**.



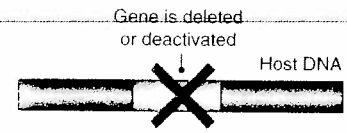
Human insulin, used to treat diabetic patients, is now produced using transgenic bacteria.



Alter an existing gene

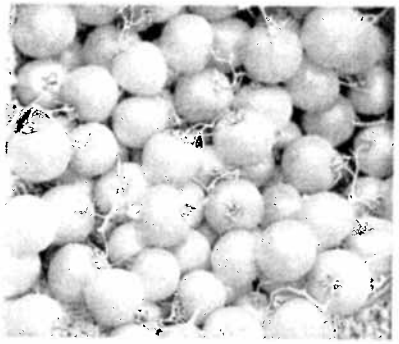
An existing gene may be altered to make it express at a higher level (e.g. produce more growth hormone) or in a different way (in tissue that would not normally express it). This method is also used for gene therapy.

Gene therapy could be used treat genetic disorders, such as cystic fibrosis.



Delete or 'turn off' a gene

An existing gene may be deleted or deactivated (switched off) to prevent the expression of a trait (e.g. the deactivation of the ripening gene in tomatoes produced the Flavr-Savr tomato).



Manipulating gene action is one way in which to control processes such as ripening in fruit.

1. Using examples, discuss the ways in which an organism may be genetically modified (to produce a GMO):

2. Explain how human needs or desires have provided a stimulus for the development of the following biotechnologies:

   (a) Gene therapy:

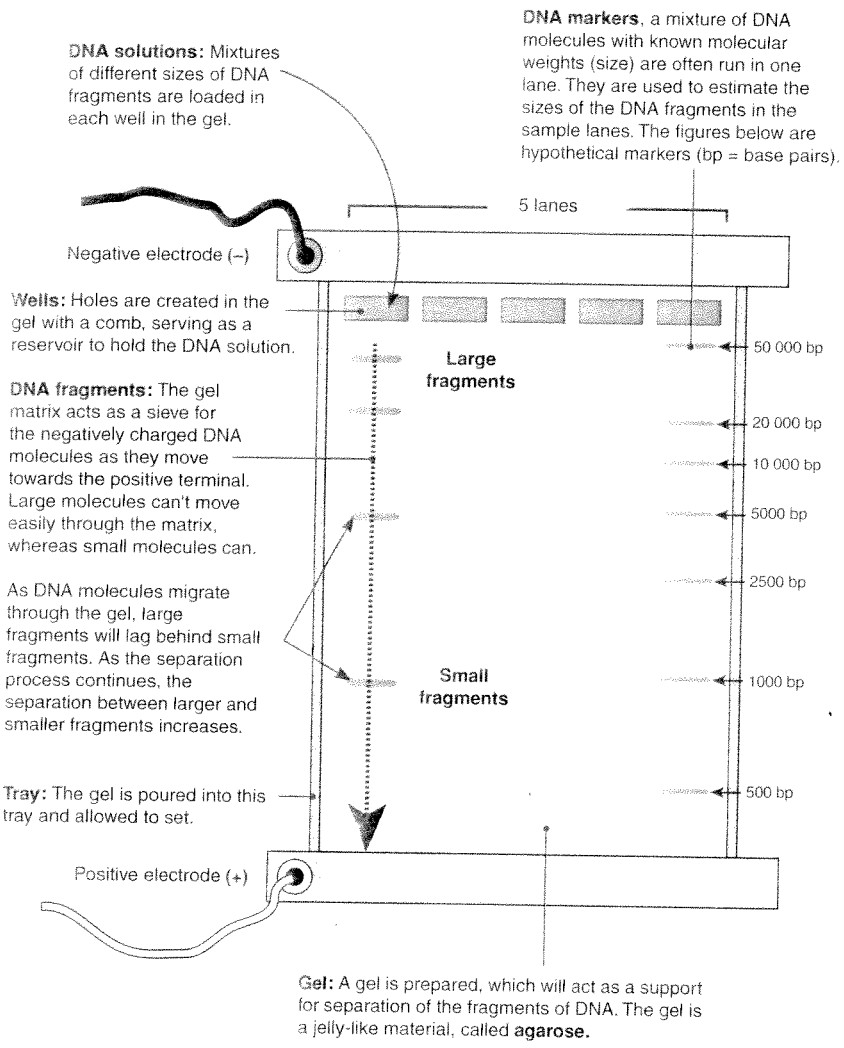
   (b) The production and use of transgenic organisms:

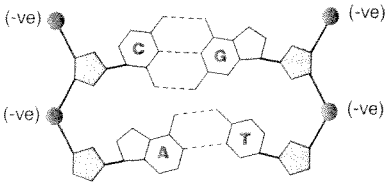
   (c) Plant micropropagation (tissue culture):

Gel Electrophoresis

Gel electrophoresis is a method that separates large molecules (including nucleic acids or proteins) on the basis of size, electric charge, and other physical properties. Such molecules possess a slight electric charge (see DNA below). To prepare DNA for gel electrophoresis the DNA is often cut up into smaller pieces. This is done by mixing DNA with restriction enzymes in controlled conditions for about an hour. Called **restriction digestion**, it produces a range of DNA fragments of different lengths. During electrophoresis, molecules are forced to move through the pores of a **gel** (a jelly-like material), when the electrical current is applied. Active electrodes at each end of the gel provide the driving force. The electrical current from one electrode repels the molecules while the other electrode simultaneously attracts the molecules. The frictional force of the gel resists the flow of the molecules, separating them by size. Their rate of migration through the gel depends on the strength of the electric field, size and shape of the molecules, and on the ionic strength and temperature of the buffer in which the molecules are moving. After staining, the separated molecules in each lane can be seen as a series of bands spread from one end of the gel to the other.

Analyzing DNA using Gel Electrophoresis





DNA is negatively charged because the phosphates (blue) that form part of the backbone of a DNA molecule have a negative charge.

Steps in the process of gel electrophoresis of DNA

1. A tray is prepared to hold the gel matrix.
2. A gel comb is used to create holes in the gel. The gel comb is placed in the tray.
3. Agarose gel powder is mixed with a buffer solution (this carries the DNA in a stable form). The solution is heated until dissolved and poured into the tray and allowed to cool.
4. The gel tray is placed in an electrophoresis chamber and the chamber is filled with buffer, covering the gel. This allows the electric current from electrodes at either end of the gel to flow through the gel.
5. DNA samples are mixed with a "loading dye" to make the DNA sample visible. The dye also contains glycerol or sucrose to make the DNA sample heavy so that it will sink to the bottom of the well.
6. A safety cover is placed over the gel, electrodes are attached to a power supply and turned on.
7. When the dye marker has moved through the gel, the current is turned off and the gel is removed from the tray.
8. DNA molecules are made visible by staining the gel with **methylene blue** or ethidium bromide which binds to DNA and will fluoresce in UV light.

1. Explain the purpose of gel electrophoresis: _____

_____

_____

2. Describe the two forces that control the speed at which fragments pass through the gel:

   (a) _____

   (b) _____

3. Explain why the smallest fragments travel through the gel the fastest: _____

_____

_____

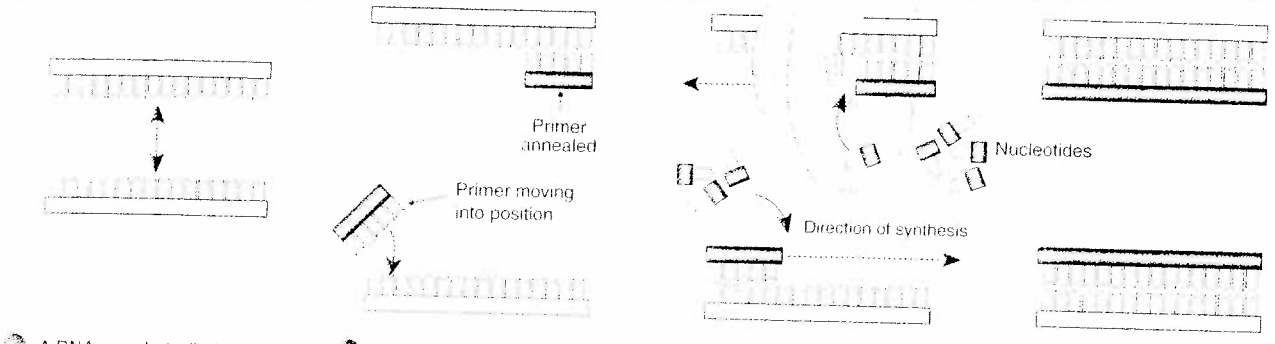
Polymerase Chain Reaction

Many procedures in DNA technology (such as DNA sequencing and DNA profiling) require substantial amounts of DNA to work with. Some samples, such as those from a crime scene or fragments of DNA from a long extinct organism, may be difficult to get in any quantity. The diagram below describes the laboratory process called **polymerase chain reaction** (**PCR**).

Using this technique, vast quantities of DNA identical to the original samples can be created. This process is often termed **DNA amplification**. Although only one cycle of replication is shown below, the following cycles replicate DNA at an exponential rate. PCR can be used to make billions of copies of DNA in only a few hours.

A Single Cycle of the Polymerase Chain Reaction

DNA polymerase: A thermally stable form of the enzyme is used (e.g. ***Taq polymerase***). This is extracted from thermophilic bacteria.



1. A DNA sample (called **target DNA**) is obtained. It is denatured (DNA strands are separated) by heating at 98°C for 5 minutes.
2. The sample is cooled to 60°C. Primers are annealed (bonded) to each DNA strand. In PCR, the primers are short strands of DNA; they provide the starting sequence for DNA extension.
3. DNA polymerase binds to the primers and, using the free nucleotides, synthesizes complementary strands of DNA.
4. After one cycle, there are now two copies of the original DNA.

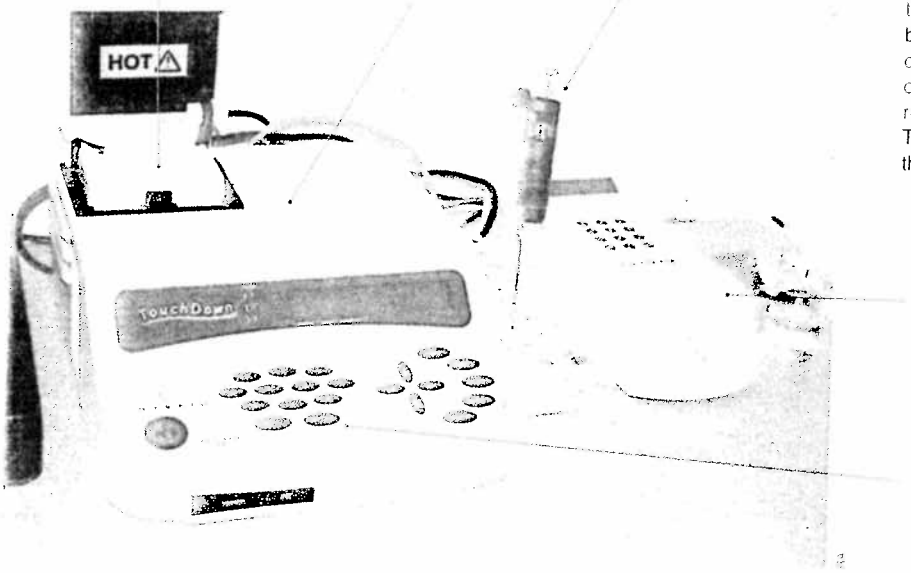
Repeat for about 25 cycles

Repeat cycle of heating and cooling until enough copies of the target DNA have been produced

Loading tray
Prepared samples in tiny PCR tubes are placed in the loading tray and the lid is closed.

Temperature control
Inside the machine are heating and refrigeration mechanisms to rapidly change the temperature.

Dispensing pipette
Pipettes with disposable tips are used to dispense DNA samples into the PCR tubes.



Thermal Cycler

Amplification of DNA can be carried out with simple-to-use machines called thermal cyclers. Once a DNA sample has been prepared, in just a few hours the amount of DNA can be increased billions of times. Thermal cyclers are in common use in the biology departments of universities, as well as other kinds of research and analytical laboratories. The one pictured on the left is typical of this modern piece of equipment.

DNA quantitation

The amount of DNA in a sample can be determined by placing a known volume in this quantitation machine. For many genetic engineering processes, a minimum amount of DNA is required.

Controls

The control panel allows a number of different PCR programmes to be stored in the machine's memory. Carrying out a PCR run usually just involves starting one of the stored programmes.

1. Explain the purpose of PCR: _____

_____

_____

2. Describe how the **polymerase chain reaction** works: _____

3. Describe three situations where only very small DNA samples may be available for sampling and PCR could be used:

   (a) _____

   (b) _____

   (c) _____

4. After only two cycles of replication, four copies of the double-stranded DNA exist. Calculate how much a DNA sample will have increased after:

   (a) 10 cycles: _____ (b) 25 cycles: _____

5. The risk of contamination in the preparation for PCR is considerable.

   (a) Describe the effect of having a single molecule of unwanted DNA in the sample prior to PCR:

   (b) Describe two possible sources of DNA contamination in preparing a PCR sample:

   Source 1: _____

   Source 2: _____

   (c) Describe two precautions that could be taken to reduce the risk of DNA contamination:

   Precaution 1: _____

   Precaution 2: _____

6. Describe two other genetic engineering/genetic manipulation procedures that require PCR amplification of DNA:

   (a) _____

   (b) _____

Applications of GMOs

Techniques for genetic manipulation are now widely applied throughout modern biotechnology: in food and enzyme technology, in industry and medicine, and in agriculture and horticulture. Microorganisms are among the most widely used GMOs, with applications ranging from pharmaceutical production and vaccine development to environmental clean-up. Crop plants are also popular candidates for genetic modification although their use, as with much of genetic engineering of higher organisms, is controversial and sometimes problematic.

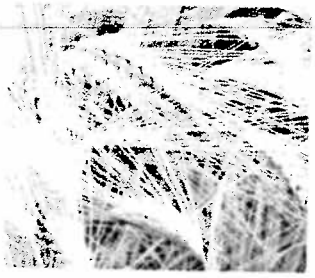
Application of GMOs

Extending shelf life

Some fresh produce (e.g. tomatoes) have been engineered to have an extended keeping quality. In the case of tomatoes, the gene for ripening has been switched off, delaying the process of softening in the fruit.

Pest or herbicide resistance

Plants can be engineered to produce their own insecticide and become pest resistant. Genetically engineered herbicide resistance is also common. In this case, chemical weed killers can be used freely without crop damage.

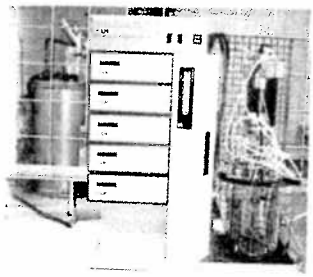


Crop improvement

Gene technology is now an integral part of the development of new crop varieties. Crops can be engineered to produce higher protein levels or to grow in inhospitable conditions (e.g. salty or arid conditions).

Environmental clean-up

Some bacteria have been engineered to thrive on waste products, such as liquefied newspaper pulp or oil. As well as degrading pollutants and wastes, the bacteria may be harvested as a commercial protein source.

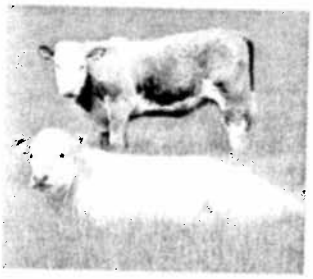


Biofactories

Transgenic bacteria are widely used to produce desirable products: often hormones or proteins. Large quantities of a product can be produced using bioreactors (above). Examples: insulin production by recombinant yeast, production of bovine growth hormone.

Vaccine development

The potential exists for multipurpose vaccines to be made using gene technology. Genes coding for vaccine components (e.g. viral protein coat) are inserted into an unrelated live vaccine (e.g. polio vaccine), and deliver proteins to stimulate an immune response.

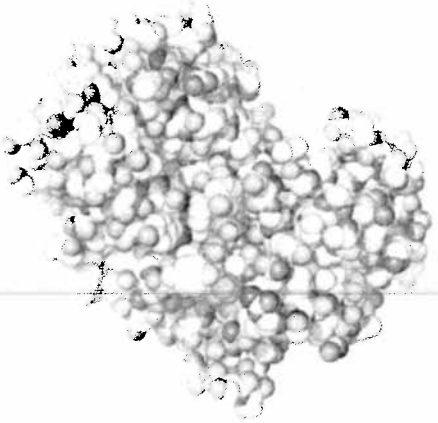


Livestock improvement using transgenic animals

Transgenic sheep have been used to enhance wool production in flocks (above, left). The keratin protein of wool is largely made of a single amino acid, cysteine. Injecting developing sheep with the genes for the enzymes that generate cysteine produces woollier transgenic sheep. In some cases, transgenic animals have been used as biofactories. Transgenic sheep carrying the human gene for a protein, α-1-antitrypsin produce the protein in their milk. The antitrypsin is extracted from the milk and used to treat hereditary emphysema.

1. In a short account discuss one of the applications of GMOs described above:

Using Recombinant Bacteria



The Issue

- **Chymosin** (also known as **rennin**) is an enzyme that digests milk proteins. It is the active ingredient in rennet, a substance used by cheesemakers to clot milk into curds.
- Traditionally rennin is extracted from "chyme", i.e. the stomach secretions of suckling calves (hence its name of chymosin).
- By the 1960s, a shortage of chymosin was limiting the volume of cheese produced.
- Enzymes from fungi were used as an alternative but were unsuitable because they caused variations in the cheese flavor.

Concept 1

Enzymes are proteins made up of amino acids. The amino acid sequence of chymosin can be determined and the mRNA coding sequence for its translation identified.

Concept 2

Reverse transcriptase can be used to synthesize a DNA strand from the mRNA. This process produces DNA without the introns, which cannot be processed by bacteria.

Concept 3

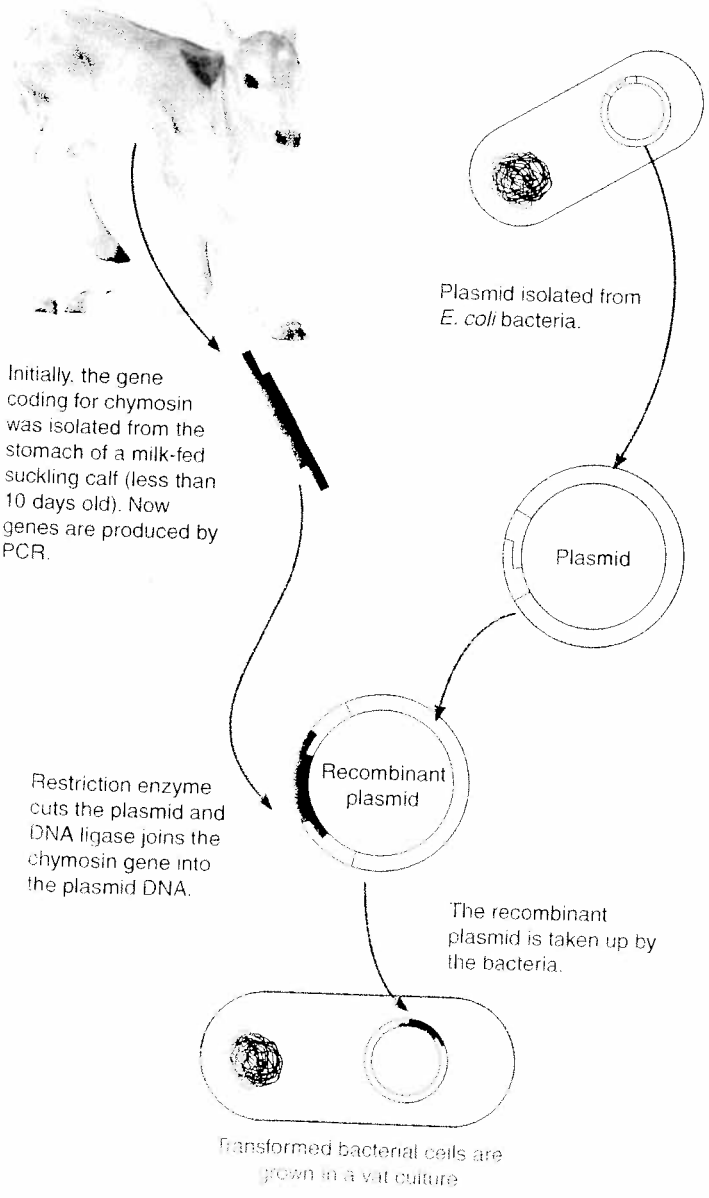
DNA can be cut at specific sites using **restriction enzymes** and rejoined using **DNA ligase.** New genes can be inserted into self-replicating bacterial **plasmids.**

Concept 4

Under certain conditions, bacteria are able to lose or take up plasmids from their environment. Bacteria are readily grown in vat cultures at little expense.

Concept 5

The protein is made by the bacteria in large quantities.



Techniques

The amino acid sequence of chymosin is first determined and the RNA codons for each amino acid identified.

mRNA matching the identified sequence is isolated from the stomach of young calves. **Reverse transcriptase** is used to transcribe mRNA into DNA. The DNA sequence can also be made synthetically once the sequence is determined.

The DNA is amplified using PCR.

Plasmids from *E. coli* bacteria are isolated and cut using **restriction enzymes.** The DNA sequence for chymosin is inserted using **DNA ligase.**

Plasmids are returned to *E. coli* by placing the bacteria under conditions that induce them to take up plasmids.

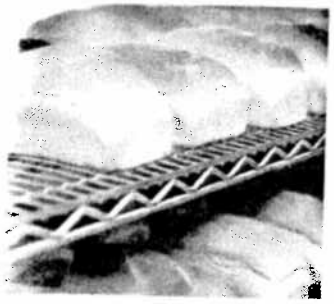
Outcomes

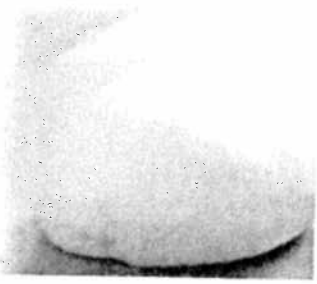
The transformed bacteria are grown in vat culture. Chymosin is produced by *E. coli* in packets within the cell that are separated during the processing and refining stage.

Recombinant chymosin entered the marketplace in 1990. It established a significant market share because cheesemakers found it to be cost effective, of high quality, and in consistent supply. Most cheese is now produced using recombinant chymosin such as CHY-MAX.

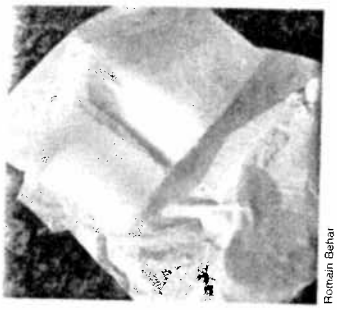
Further Applications

A large amount of processing is required to extract chymosin from *E.coli*. There are now a number of alternative bacteria and fungi that have been engineered to produce the enzyme. Most chymosin is now produced using the fungi ***Aspergillus niger*** and ***Kluyveromyces lactis***. Both are produced in





Enzymes from GMOs are widely used in the baking industry. Maltogenic alpha amylase from *Bacillus subtilis* bacteria is used as an anti-staling agent to prolong shelf life. Hemicellulases from *B. subtilis* and xylanase from the fungus *Aspergillus oryzae* are used for improvement of dough, crumb structure, and volume during the baking process.



Romain Behar

Lipase from *Aspergillus oryzae* is used in processing of palm oil to produce low cost cocoa butter substitutes (above), which have a similar 'mouth feel' to cocoa butter.

Dual Frog

Acetolactate decarboxylase from *B. subtilis* is one of several enzymes used in the brewing industry. It reduces maturation time of the beer by by-passing a rate-limiting step.

1. Describe the main use of chymosin: _____

2. What was the traditional source of chymosin? _____

3. Summarize the key concepts that led to the development of the technique for producing chymosin:

   (a) Concept 1: _____

   (b) Concept 2: _____

   (c) Concept 3: _____

   (d) Concept 4: _____

   (e) Concept 5: _____

4. Discuss how the gene for chymosin was isolated and how the technique could be applied to isolating other genes:

5. Describe three advantages of using chymosin produced by GE bacteria over chymosin from traditional sources:

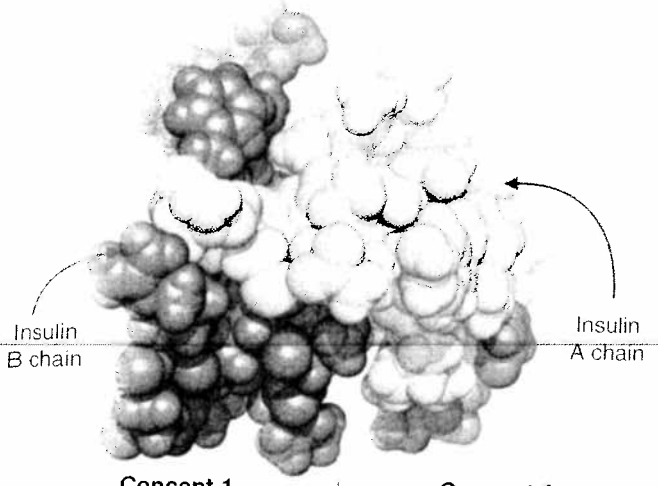
   (a) _____

   (b) _____

   (c) _____

6. Explain why the fungus *Aspergillus niger* is now more commonly used to produce chymosin instead of *E. coli*:

Production of Insulin



The Issue

- **Type 1 diabetes mellitus** is a metabolic disease caused by a lack of **insulin**. Around 25 people in every 100,000 suffer from type 1 diabetes.
- It is treatable only with injections of insulin.
- In the past, insulin was taken from the pancreases of cows and pigs and purified for human use. The method was expensive and some patients had severe allergic reactions to the foreign insulin or its contaminants.

Concept 1

DNA can be cut at specific sites using **restriction enzymes** and joined together using **DNA ligase**. New genes can be inserted into self-replicating bacterial **plasmids** at the point where the cuts are made.

Concept 2

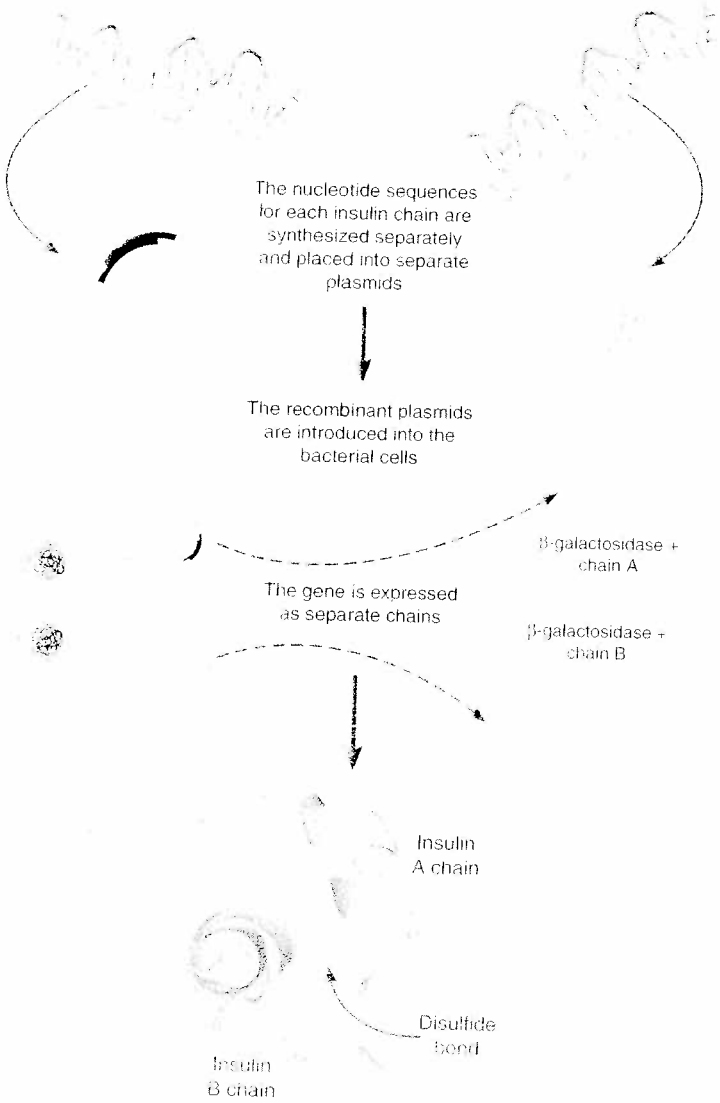
Plasmids are small, circular pieces of DNA found in some bacteria. They usually carry genes useful to the bacterium. *E. coli* plasmids can carry promoters required for the transcription of genes.

Concept 3

Under certain conditions, Bacteria are able to lose or pick up plasmids from their environment. Bacteria can be readily grown in vat cultures at little expense.

Concept 4

The DNA sequences coding for the production of the two polypeptide chains (A and B) that form human insulin can be isolated from the human genome.



Techniques

The **gene** is **chemically synthesized** as two nucleotide sequences, one for the **insulin A chain** and one for the **insulin B chain**. The two sequences are small enough to be inserted into a plasmid.

Plasmids are extracted from *Escherichia coli*. The gene for the bacterial enzyme **β-galactosidase** is located on the plasmid. To make the bacteria produce insulin, the insulin gene must be linked to the **β-galactosidase** gene, which carries a promoter for transcription.

Restriction enzymes are used to cut plasmids at the appropriate site and the A and B insulin sequences are inserted. The sequences are joined with the plasmid DNA using **DNA ligase**.

The **recombinant plasmids** are inserted back into the bacteria by placing them together in a culture that favors plasmid uptake by bacteria.

The bacteria are then grown and multiplied in vats under carefully controlled growth conditions.

Outcomes

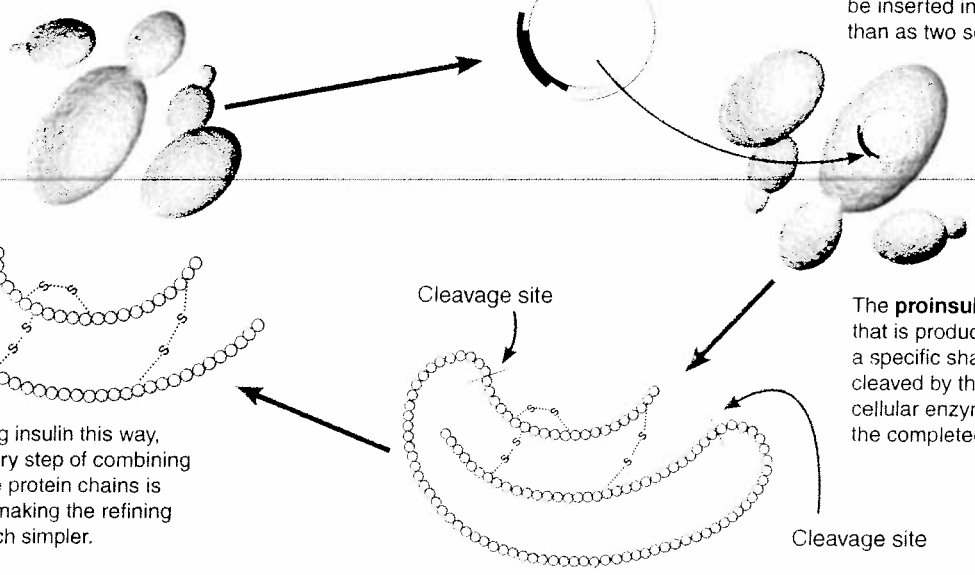
The product consists partly of β-galactosidase, joined with either the A or B chain of insulin. The chains are extracted, purified, and mixed together. The A and B insulin chains connect via **disulfide cross linkages** to form the functional insulin protein. The insulin can then be made ready for injection in various formulations.

Further Applications

The techniques involved in producing human insulin from genetically modified bacteria can be applied to a range of human proteins and hormones. Proteins currently being produced include human growth hormone, interferon, and factor VIII.

Insulin production in *Saccharomyces*

Yeast cells are **eukaryotic** and hence are much larger than bacterial cells. This enables them to accommodate much larger plasmids and proteins within them.



The gene for human insulin is inserted into a plasmid. The yeast plasmid is larger than that of *E.coli*, so the entire gene can be inserted in one piece rather than as two separate pieces.

By producing insulin this way, the secondary step of combining the separate protein chains is eliminated, making the refining process much simpler.

The **proinsulin** protein that is produced folds into a specific shape and is cleaved by the yeast's own cellular enzymes, producing the completed insulin chain.

1. Describe the three major problems associated with the traditional method of obtaining insulin to treat diabetes:

   (a) _____

   (b) _____

   (c) _____

2. Explain the reasoning behind using *E. coli* to produce insulin and the benefits that GM technology has brought to diabetics:

   _____

   _____

   _____

   _____

3. Explain why, when using *E. coli*, the insulin gene is synthesized as two separate A and B chain nucleotide sequences:

   _____

   _____

4. Why are the synthetic nucleotide sequences ('genes') 'tied' to the β-galactosidase gene? _____

   _____

   _____

5. Yeast (*Saccharomyces cerevisiae*) is also used in the production of human insulin. Discuss the differences in the production of insulin using yeast and *E. coli* with respect to:

   (a) Insertion of the gene into the plasmid: _____

   _____

   _____

   (b) Secretion and purification of the protein product: _____

   _____

   _____