F215 Control, genomes and environment

Unit 6: Biotechnology and gene technologies

Plant Clones

A **clone** is an exact copy of an organism, and by this we mean genetically identical. Plants can reproduce **asexually** by producing **runners** (shoots which run along the ground and develop buds which grow as full, genetically identical plants), which means all offspring are clones of the parent. Advantages and disadvantages of asexual reproduction:

- ✓ it is quick, allowing organisms to reproduce rapidly, taking advantage of resources in their environment
- ✓ it can also be completed where sexual reproduction fails or is not possible
- ✓ all offspring have the same genetic information which enables them to survive in their environment
- ✗ it does not produce any genetic variation, so any genetic parental weaknesses will be passed on, and all members of the species will be susceptible to the same selection pressures
- ✗ likewise, with no genetic variety, this means that adaptations are reduced

Vegetative propagation refers to the production of structures which can grow into individual new organisms. There are both natural and artificial methods to this:

- producing **root suckers** (or *basal sprouts*) following the death or destruction of a parent plant, which are created from existing **meristem tissue** a short period after the death of the parent: this can help them to survive disease or other catastrophes, such as burning (*English elms* use this method, for example whilst alive an adult elm will grow root suckers, and if the adult develops **Dutch elm disease** healthy clones will still grow)
- **runners** run along the surface of the ground and have buds which can develop into adult plants
- specialised underground stems called **tubers** can produce clones in other places

Artificial vegetative propagation can take three main forms:

- taking **cuttings** from living plants, from a section that can grow new cloned plants when treated with hormones
- **grafting** – this involves taking a short section of a woody plant and grafting it onto an already-growing root or stem, known as a **rootstock**, which will grow into a clone of the parent plant (despite not being identical to the rootstock)
- but if this is to be done on a mass scale, **tissue culture** will be needed, using a technique called **micropropagation**:
  - a small piece of meristem tissue (the **explant**) is taken and placed in a growth medium with nutrients
  - cells divide (but do not specialise), producing a batch of undifferentiated cells, called the **callus**
  - after a few weeks, single callus cells can be separately placed on a medium with growth hormones
  - after some weeks of growth these can be transferred to another medium which encourages root growth
  - growing plants can then soon be transferred to a **greenhouse** where they can fully grow

Animal Clones

Only **totipotent** animal cells are capable of differentiating and becoming any cell. They're capable of **specialisation** and switching certain genes within the genome *on* or *off*. A **cloned** animal is one which contains the exact same genetic information to the parent animal, and has been produced using only the parent's genetic material

By **embryonic division**, cells from a developing embryo are divided, with each batch being capable of developing into the adult organism (essentially created artificial identical twins). The embryo is grown ***in vitro*** and then split, where the embryonic fragments are implanted into surrogate mothers, and continue to grow

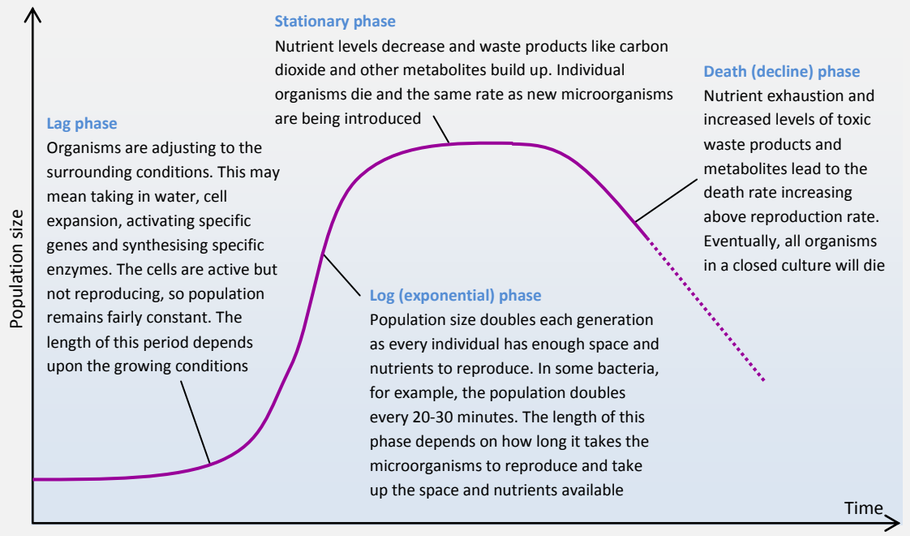
By **somatic nuclear transfer**, the nucleus of a **somatic cell** (body cell, non-sex cell) is transferred into an **enucleated** (having had the nucleus removed) egg cell. This means that the entire genetic material from the donor adult animal is reproduced in the embryo which is cultured in the tied oviduct of a third animal, and finally transferred to a fourth (the surrogate) where it fully grows and will produce a child genetically identical to the donor animal

Both of these methods and the methods of cloning plants are reproductive, but recently interest into the area of **non-reproductive cloning** has sparked research which means in the future generating entirely new organisms may be possible. This could be used to help repair damaged tissues and organs, ending long donor organ waiting lists

Biotechnology

The term **biotechnology** describes the use of technological processes which use living organisms. Often biotechnology has commercial applications, and mostly makes use of **microorganisms**, such as bacteria and fungi. This is because they have quick **generation times** (a new generation can be produced in around 20 minutes with some bacteria) and can be **genetically engineered** for industrial use

A **culture** is a group of one type of microbe which has been grown purposefully under controlled conditions. A **closed culture** takes place in a vessel which is totally sealed from the environment. Closed cultures follow a standard **growth curve**:



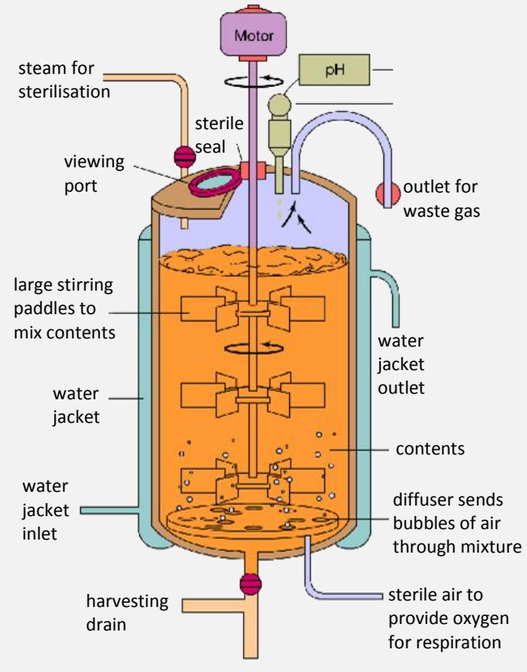
During the exponential phase (so when growing conditions are favourable), microbes produce **primary metabolites** – substances essential for their growth, such as amino acids, proteins and enzymes. During the stationary phase (when the conditions are less favourable), they produce **secondary metabolites** – which are not essential to their growth but may still be useful, such as *penicillin*

Secondary metabolites are often harvested from cultures grown in **industrial fermenters** (below). These are regulated in terms of:

- **pH** – controlled for enzyme efficiency
- water jacket controls **temperature**
- stirring paddles keep microbes in contact with fresh medium
- sterilised often by **superheated steam**

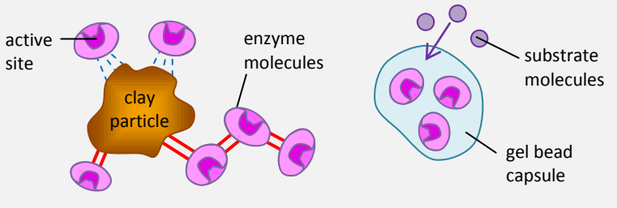
Batch cultures produce metabolites in batches and are allowed to reach the *death phase* of the growth curve. When this stage is reached, the vessel is emptied and new microbes are cultured and new medium used. **Continuous cultures** require constant introduction of fresh growth medium, and frequent removal of waste products, but the cultures are allowed to grow continually, producing more and more metabolites

It is important to prevent contamination to the culture, as this can be expensive, wasting much time and investment into the culture. The process of preventing contamination is known as **asepsis**. Methods of asepsis include washing, sterilising and disinfecting apparatus, sterilising the growth medium, and having fine filters on inlets and outlets to the vessel to prevent unwanted immigration of microbes



Biotechnological fermentation usually requires the use of **isolated enzymes** to catalyse reactions. These are extracted in a process called **downstream processing**, which refers to the harvesting of a certain substance from a fermentation cycle. The isolated enzymes are mass produced, and may be **immobilised** to ensure they are not removed when metabolites are harvested (so they are recycled, can be used again and again – remaining inside the vessel). This saves time and money and increases efficiency. Methods are:

- **adsorption** (left diagram, blue bonds) involves enzymes mixing with an *immobilising support material* such as *clay* and form **hydrophobic** and **ionic bonds** with the material, clumping the enzymes together (although the bonds are weak and prone to breaking, enzyme efficiency with adsorption is still high
- **covalent bonding** (left diagram, red bonds) involves enzymes covalently bonding to the support material as well as each other, again clumping the isolated enzymes together (also forming long chains of covalently-bonded enzymes), and these bonds are more tough and less likely to break
- **entrapment** (right diagram) means trapping the isolated enzymes within a gel or cellulose bead so that substrate molecules can pass through the barrier and enter the active site, but enzymes cannot leave as they are too big: although this is not often effective as substrates have to pass the casing



Sequencing the Genome: BACs

Before the development of modern *automated sequencing*, lengths of DNA could be sequences using bacteria. The lengths would be **sheared** into small fragments of around 100,000 bases, and implanted into **bacterial artificial chromosomes** (BACs) and taken up by *E. coli* cells. As the cells grow by culture, **clone libraries** (many copies of the target length) are produced

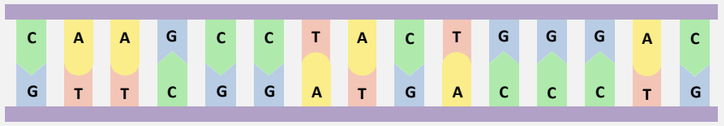
To sequence a length of DNA in a BAC section:

- the DNA is extracted and various **restriction enzymes** are used to cut the DNA lengths into different samples
- the fragments are separated using a process called *electrophoresis*
- each fragment is then sequenced using an automated process (this is only made possible with the use of modern techniques: see *Sanger sequencing*)
- computer programmes then can compare overlapping regions from the cuts made by the different restriction enzymes in order to reassemble the whole BAC section sequence

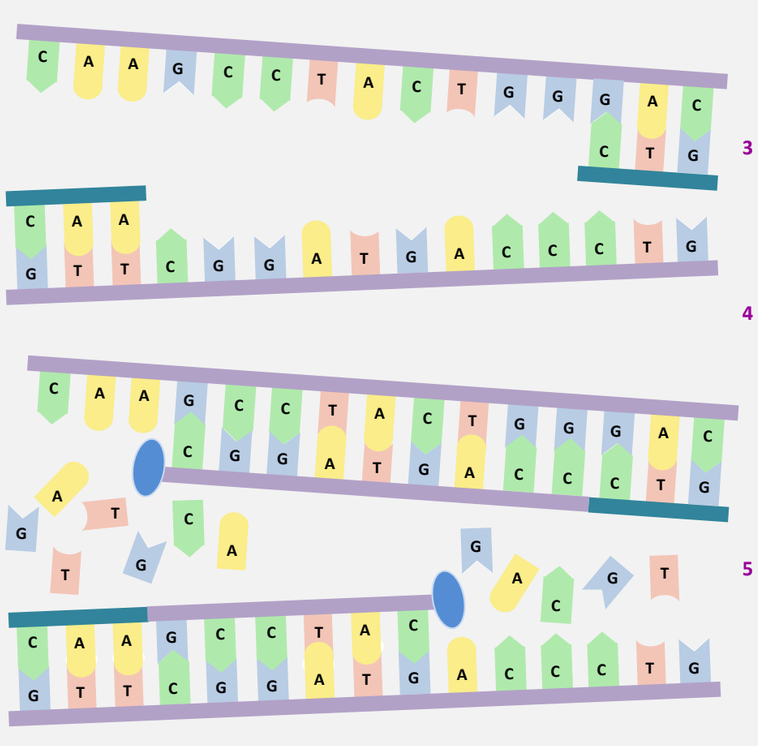
Sequencing the **genome** of an animal (entire genetic material) has many uses, such as allowing us to identify universal codings, which must code for molecules which as important to all life, and identifying sequences unique to pathogenic organisms, which can lead to the development of more successful drugs

Polymerase Chain Reaction

The **polymerase chain reaction** (PCR) is a method of duplicating a target length of DNA. In just 25 cycles of the reaction, the process produces over 33 million copies of a single piece of DNA. The ingredients required for the PCR 'broth' are: the target length of DNA, DNA **primers** (short lengths of DNA which match the start of the target length), free nucleotides, and **DNA polymerase**



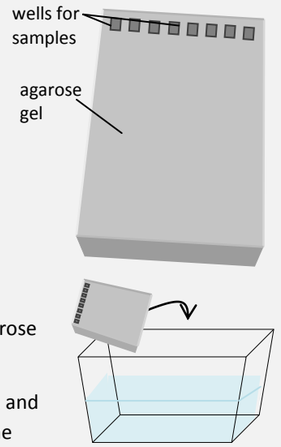
1. The process begins with the target length to be copied
2. The mixture is heated to **95°C** for a short period, to break down the hydrogen bonds holding the two strands together, giving two separated strands of DNA
3. Then the mixture is cooled to **55°C** to allow the primers to **anneal** (bond) to the exposed bases at the end of each sequence – hydrogen bonding
4. The mixture is heated slightly to **72°C** to provide the **taq polymerase** enzyme (a special form of DNA polymerase) its optimal temperature, which builds a complementary strand for each single strand, using the free nucleotides
5. When taq polymerase reaches the end of the sequence, it detaches and the cycle repeats to give **exponential** duplication as the number of copies you have doubles each cycle



The enzyme taq polymerase comes from a bacterium which lives in hot springs (*Thermus aquaticus*) and so has an optimal temperature of 72°C and can survive the extreme temperatures of the reaction

Electrophoresis

Electrophoresis is a process which can be used to separate fragments of DNA by length, and is accurate enough to differentiate strands different in length by only one base pair. The process uses a gel plate made of **agarose gel** covered in a **buffer solution**. Whilst the gel sets, a comb is inserted to create wells to place the DNA samples in



1. DNA samples are treated with **restriction enzymes** to cut them to fragments
2. the fragments are placed into the wells at the end of the gel plate where the negative **electrode** will be
3. the plate is immersed into a tank filled with buffer solution
4. an electric current is passed through the tank for a fixed amount of time (usually one hour)
5. due to the **phosphoryl groups** of the sugar-phosphate backbone in DNA molecules, the samples have negative **electronegativity** and so are attracted to the other end of the plate, where the positive electrode is, so the molecules move along the plate to the other end

The shorter fragments are able to move more quickly than the longer ones, as the longer ones get caught up in the agarose gel more. Therefore the shorter fragments have moved further than the longer ones at the end of the fixed time

A process called **Southern blotting** may then be used to examine the results. A **nylon sheet** is placed over the gel plate, and then paper towels are placed on top, and the samples are *blotted*. The DNA fragments are drawn up by **capillarity** to the nylon sheet (remaining in order by length)

The samples will be treated with **radioactive markers** beforehand, so that once transferred to the nylon sheet, a piece of photographic film can be placed on top to show the position of each fragment

DNA Probes

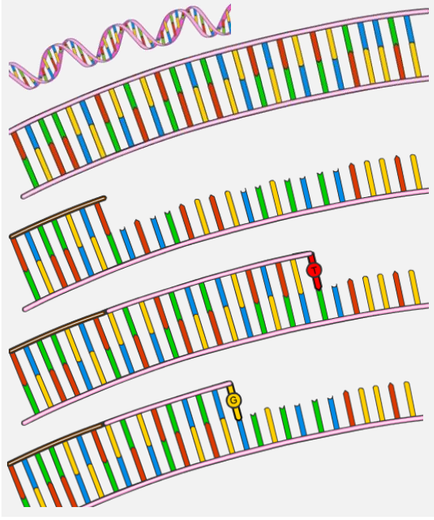
A **DNA probe** is a short, single-stranded length of DNA (usually no more than 50 bases long) which can be used to test for the presence of certain lengths of DNA (as the piece being tested for will be complementary to the probe). A probe can be used in a number of ways:

- using **radioactive isotopes** on the phosphoryl groups so that it is identifiable under photographic film (as with Southern blotting)
- using **fluorescent markers** which emit a certain light when exposed to ultraviolet light (as with automated sequencing)
- using a specific '**antigen**' which is complementary to a specific known '**antibody**' – which itself will be labelled radioactively or fluorescently

DNA probes can be useful for finding particular genes which are needed for genetic engineering techniques, and for indentifying the same gene from the genomes of two different organisms. This can be used to compare genomes so that we can learn more about what the genetic code really codes for and what is unique to certain organisms. DNA probes can also be used to identify the presence or absence of an allele for a particular genetic disease, which helps to detect it which allows for quicker treatment

Sanger Sequencing

Also known as the **chain termination method** or **automated sequencing**, this method called **Sanger sequencing** is the most common technique in use nowadays to sequence an unknown length of DNA. For this process, many thousands of copies of the target length are needed: these are produced by *polymerase chain reaction*. Also required is **DNA polymerase**, free nucleotides and DNA **primers** which match the beginning of the sequence



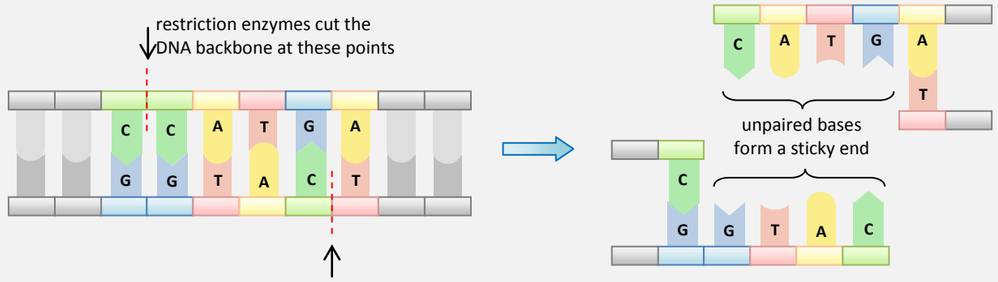
1. A target length to be sequenced is taken, which is heated to separate the two strands by breaking their hydrogen bonds (there is only one strand we need to sequence)
2. The reaction is initiated at either end of the sequence, where a primer anneals to exposed bases
3. DNA polymerase then begins to build a complementary strand, from where the primers end, using free nucleotides in the mixture
4. In the mixture, there are some modified nucleotides called **dideoxynucleoside triphosphate** molecules (ddNTP) which carry a fluorescent marker and when DNA polymerase anneals one of these to the strand (they have equal affinity and bind as normally as nucleotides) it throws the enzyme off and the strand stops being built there
5. DNA polymerase builds complementary strands on all of the copies of the DNA samples, so you end with a large number of fragments all terminating with a ddNTP molecule at a different location (so different length fragments)

The fragments are first separated using *electrophoresis* and *Southern blotting*, and then are run through a computer – this is the automated sequencing bit – where a laser detects where each fragment terminates, as it can read the fluorescent markers at the end of each chain. The computer software can then sequence the whole length based on where the different lengths terminate

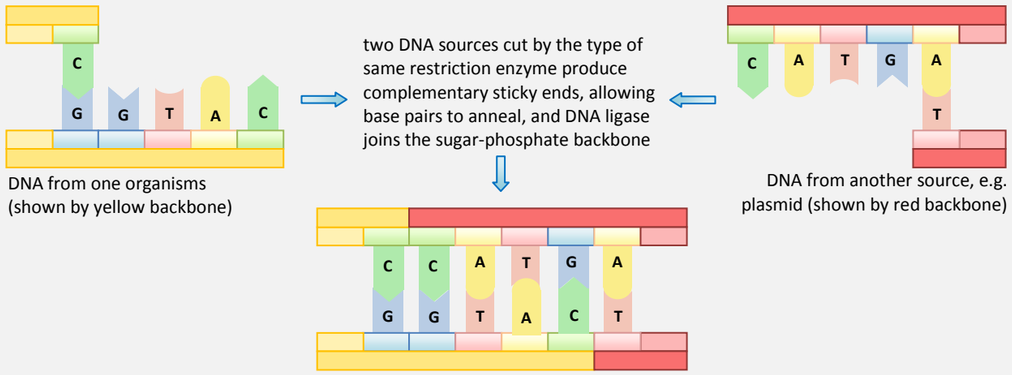
Genetic Engineering

Recombinant DNA technology, or **genetic engineering**, involves combining DNA from different sources. A target gene can be obtained, usually being identified using a **DNA probe** and then cut using *restriction enzymes*, and then placed in a **vector**, such as a bacterial plasmid or into viral DNA. This is then moved into the recipient cell (some bacteria can just take up plasmids, but sometimes **liposomes** may be used), and the host takes on the DNA and should express the gene by protein synthesis

Restriction enzymes cut through the DNA sugar-phosphate backbone at specific points called **restriction sites**. Particular types of restriction enzyme will only cut through one particular site (determined by the base sequence of the site). When the enzyme does this is leaves exposed bases on either strand, which are called **sticky ends**



The enzyme **DNA ligase** repairs the sugar-phosphate backbone, sealing the molecule and reforming the double-helical structure. Only DNA fragments cut with the same restriction enzyme can be sealed using DNA ligase as only then will they have complementary sticky ends



Bacterial Engineering

A bacterial **plasmid** is a DNA molecule entirely separate from the main bacterial chromosome. They are often used as a vector where genes from other sources are inserted to the plasmid so that it can be cultured. The result is **recombinant DNA**. Placing the target gene in a mixture with lots of plasmids (cut open with restriction enzymes) and supplying DNA ligase will cause some plasmids to take up the target gene, although most will simply use DNA ligase to reseal their plasmids shut. The plasmids are then mixed with bacteria, some of which will take up the recombinant plasmid and be **transformed bacteria**

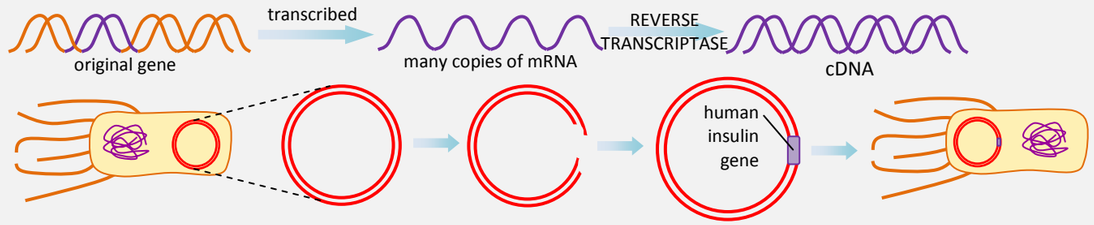
Bacteria can take up DNA from their external environment. One way they do this is **conjugation** – conjugational tubes form between bacterial cells, and plasmids unwind and move from bacterium to bacterium. Essentially, genetic material is swapped and replicated between cells. This can be problematic as plasmids code for resistance to many antibiotics, and so by conjugation, resistance to our antibiotics is spread

Genetic Manipulation Leading to Xenotransplantation

Research into genetic engineering has led to strong developments in the area of **xenotransplantation**, the use of engineering animals to grow organs for human transplantation. It is estimated that 60% of patients on donor organ waiting lists die before receiving their organ, so this would be a massive breakthrough with more research. Recently, experiments using pigs has allowed the growth of tissue in pigs using human enzymes, as scientists have manipulated these pigs (in particular, an enzyme called **α-1,3 transferase** which is responsible for graft rejection in transplantation), and so these experiments are showing little or no immunological response from the pigs. This suggests it may be possible that pig organs for transplants

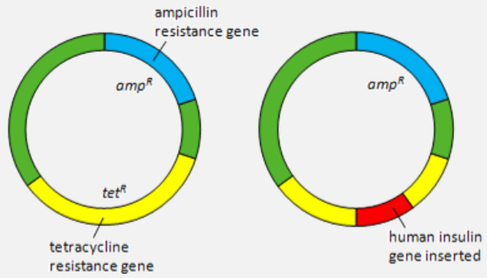
Manufacturing Human Insulin

1. The **mRNA strand** coding for human insulin is isolated and the enzyme **reverse transcriptase** is used to produce a single-stranded DNA copy of the gene (complementary to the mRNA sequence)
2. **DNA polymerase** then builds a complementary strand forming a double-stranded copy of the insulin gene called **cDNA**
3. A restriction enzyme is used to cut open plasmids from the *E. coli* bacterium, and they are mixed with the cDNA to take up the gene
4. A culture of *E. coli* are then mixed with the plasmids (empty and with the gene) and grown to mass produce insulin



It is difficult to tell which plasmids have taken up the cDNA, and also which bacteria have taken up a plasmid at all, let alone the recombinant plasmid. Therefore, using **replica plating**, the bacteria are grown in a culture on a plain nutrient agar, so they can all grow. The bacteria are then moved to an **ampicillin** agar medium, and finally onto a **tetracycline** medium

Those which have taken up the insulin gene will *not* be resistant to tetracycline, because the gene is inserted in the middle of the resistance site for the antibiotic, but they will still be resistant to the antibiotic ampicillin. Therefore, those which grow on the ampicillin plate but *not* the tetracycline plate have the cDNA plasmid



Golden Rice™

Golden Rice has been developed to help those who are risk of suffering from a **vitamin A** deficiency. Rice plants contain the gene that codes for **beta-carotene**, a precursor to the molecule **retinol** (vitamin A). However, beta-carotene does not form naturally in the bit of the rice plant that we eat (called the **endosperm**)

The endosperm areas of the plant do, however, contain most of the metabolic pathway to produce beta-carotene, but they lack two enzymes which enable its production: because from an evolutionary point of view, the rice plant has no need for the molecule in that area. The two enzymes are **phytoene synthetase** and **Crt-1 enzyme**. The genes coding for these enzymes have been extracted from other sources and inserted into the DNA of the endosperm of rice plants (*phytoene synthetase* comes from a daffodil, and *Crt-1 enzyme* comes from a bacterium)

With these two enzymes, Golden Rice is able to produce beta-carotene, which means those who do not have other access to retinol can obtain it from beta-carotene. Golden Rice is described as **biofortified** because it contains higher levels of certain beneficial substances that it naturally would. The product is currently undergoing food safety investigations and full crop trials are expected to run in 2012

Gene Therapies

When gene technologies are used to treat genetic disorders, this is known as **gene therapy**. Recent research developments have suggested possible further uses in the future for gene therapies, including the use of **RNA interference** (RNAi) which could silence undesirable genes by binding to their mRNA. At present this is only used to combat the AIDS **cytomegalovirus**

Somatic cell gene therapy involves treating *somatic cells* (non-sex cells). This usually means inserted genes to the DNA (so adding to the genome, rather than 'replacing' genes), which can override certain faulty genes and allow proper gene expression. This therapy may also involve targeting cancerous cells and stimulating them to express genes which produce antigens, making the cells vulnerable to attack from the immune system. This causes the patient's own immune system to attack its malignant tumours

Germline cell gene therapy involves introducing genes or DNA to **germline cells** (sex cells). This can be prior to fertilisation, either to the sperm, egg, or can be to the fertilised zygote or developing embryo. Introduction to such cells means that as they replicate, the offspring will have cells which *all* contain the target DNA (not the case with somatic treatment). Also, somatic treatment needs to be carried out more often, as this also means the inserted genes are not transferred by mitotic division. Whilst germline cell therapy seems more beneficial, and is mostly more effective, it is more controversial, as it means altering the genome of an unborn child, which raises ethical concerns (you cannot guarantee that the therapy won't have unwanted harmful effects on the child when they are born)