6.6 Genetic engineering

Using bacteria and genetic engineering to produce insulin and Golden Rice™

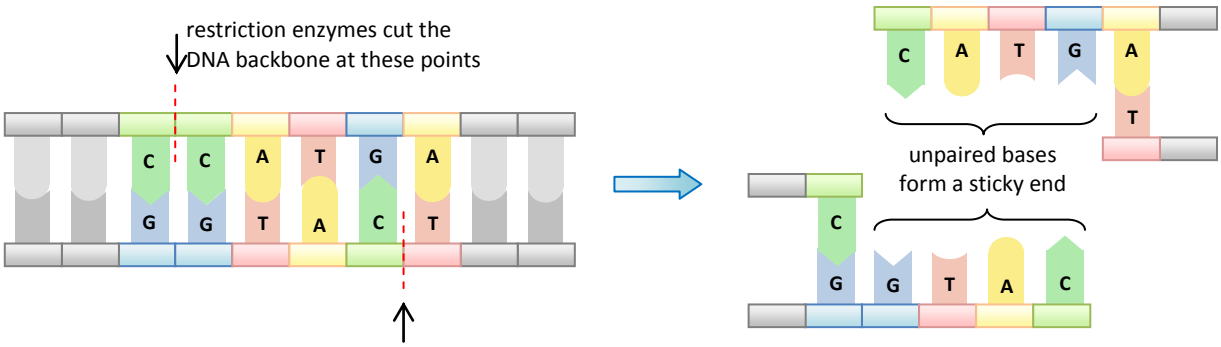
Recombinant DNA technology

The process of genetic engineering is usually named **recombinant DNA technology** by geneticists and scientists due to the nature of the technology: it involves combining DNA from different sources or different organisms, in a single organism. The resultant DNA, where fragments from different sources join, is called **recombinant DNA** (rDNA). The steps involved in genetic engineering are outlined below:

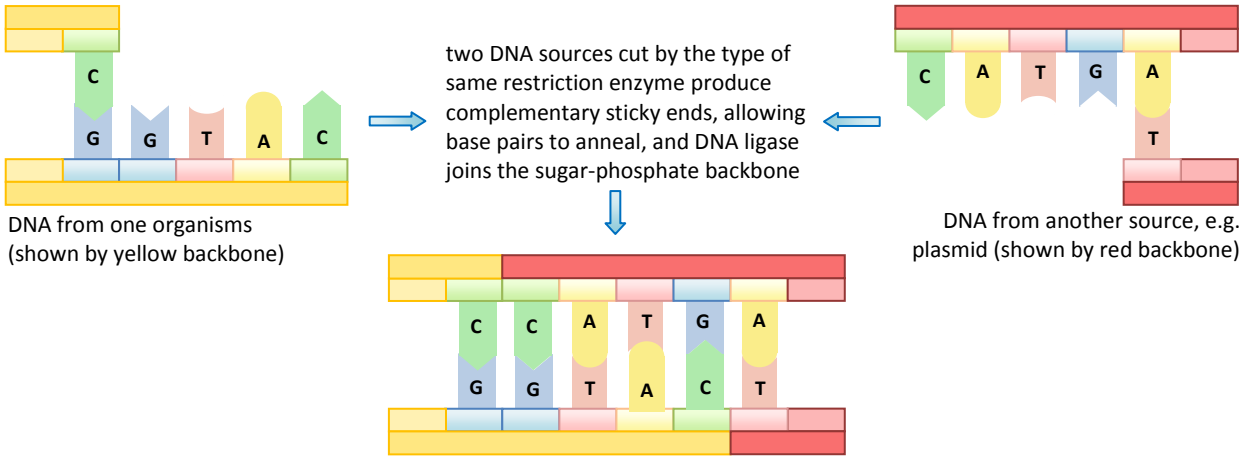
1. The required gene is obtained
   *The gene in question is usually obtained through getting the mRNA strand which codes for the gene (for example, the gene for insulin production comes from the mRNA strand from β-cells in the islets of Langerhans), and can usually be located using a **DNA probe** on DNA fragments*
2. A copy of the gene is placed in a **vector**
   *A vector is a means of delivering a gene into a cell, and a carrier into which the required gene is inserted, resulting in recombinant DNA: common vectors include bacterial **plasmids**, viral DNA and liposomes*
3. The vector carries the gene to the recipient cell
   Once packaged in a vector, the gene forms quite a large molecule which does not easily cross the membrane of a cell, and so methods to get the gene into the target cells include:
   a. **electroporation** – a high-voltage shock is administered to disrupt the cell surface membrane
   b. **microinjection** – DNA can be injected using a very fine micropipette into the host cell's nucleus
   c. **liposomes** – DNA can be wrapped around lipid molecules, which are fat-soluble and can cross the membrane
4. Via protein synthesis, the recipient expresses the gene

Restriction and ligase enzymes

Genetic engineering involves the use of cutting up and sticking together various bits of DNA. **Restriction enzymes** are used to cut through DNA at specific points. These enzymes were first lifted from bacteria, which use them as a natural defence mechanism against viral pathogens. A particular restriction enzyme will cut DNA at specific points where particular base sequences occur (called a **restriction site**), usually under ten bases long. Generally, restriction enzymes catalyse *hydrolysis* reactions which break the sugar-phosphate backbone of DNA molecules at specific points. The cutting of DNA in places like this leaves **sticky ends** as there are unpaired and exposed bases along the molecule.



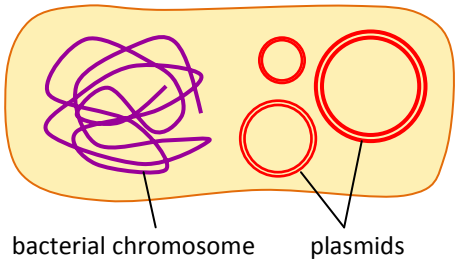
When separate fragments are to be joined together, an enzyme called **DNA ligase** is used to catalyse the *condensation* reaction joining the sugar-phosphate backbones of the double helix together. This is the same enzyme that has this function during semi-conservative DNA replication. Only fragments which were cut with the same restriction enzyme can be joined by DNA ligase – because only then will they have complementary sticky ends, allowing the bases to pair up and form hydrogen bonds, so DNA ligase can seal the backbone. The result is recombinant DNA. The diagram at the top of the following page shows how sticky ends rejoin and the backbone is sealed with DNA ligase.



Bacterial genetic engineering

A **plasmid** is a DNA molecule which is entirely separate from the chromosomal DNA found in bacteria (and a very small amount of eukaryotes). Bacteria often have their chromosomal DNA (called a *nucleoid*) and then the plasmid which is kept separate. The diagram shows a bacterium with these features.

The vast majority of the time, genetic engineering uses bacterial plasmids as the vector to place a gene in once it has been identified. Restriction enzymes are used to cut a gene from a DNA molecule, and then it is inserted into the plasmid. Plasmids are usually circular DNA molecules which contain genetic coding for resistance to antibiotic chemicals.

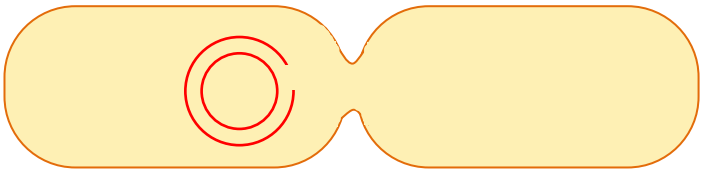


When quantities of the plasmid and the gene are mixed together with DNA ligase enzymes, some of the plasmids will combine with the gene, sealing the gene into the plasmid and forming a **recombinant plasmid**.

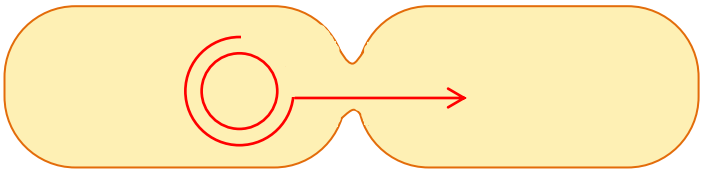
Whilst in this bacterial broth most of the plasmids will simply use DNA ligase to reseal their own cut plasmids, some of them will take on board the desired gene. The plasmids with the gene are then mixed with bacterial cells, some of which will take up the recombinant plasmid, although efficiency is under 1% of bacterial cells actually doing so. Those which do, however, are said to be **transformed bacteria**. The result is that the bacteria contain new DNA, and so are described as being **transgenic** (a term used to describe an organism which has added DNA due to genetic engineering).

Conjugation

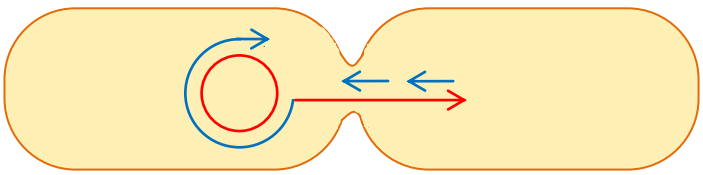
Bacteria are capable of a process known as **conjugation**, where genetic material may be exchanged. In this process, copies of plasmid DNA are passed between bacteria. Since plasmids are often carrying genes for resistance to antibiotics this is of concern as it speeds up the spread of resistance between bacterial populations.



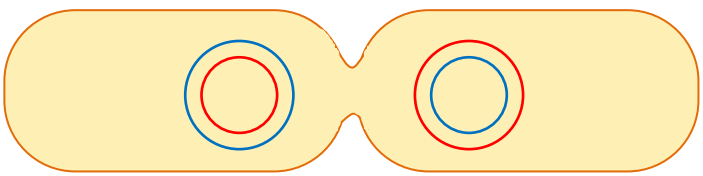
a Conjugation tube forms between a *donor* and a *recipient* and an enzyme creates a **nick** in the plasmid



b Plasmid DNA starts replicating, and the free DNA strand begins to move through the tube



c In the recipient cell, replication starts on the transferred DNA

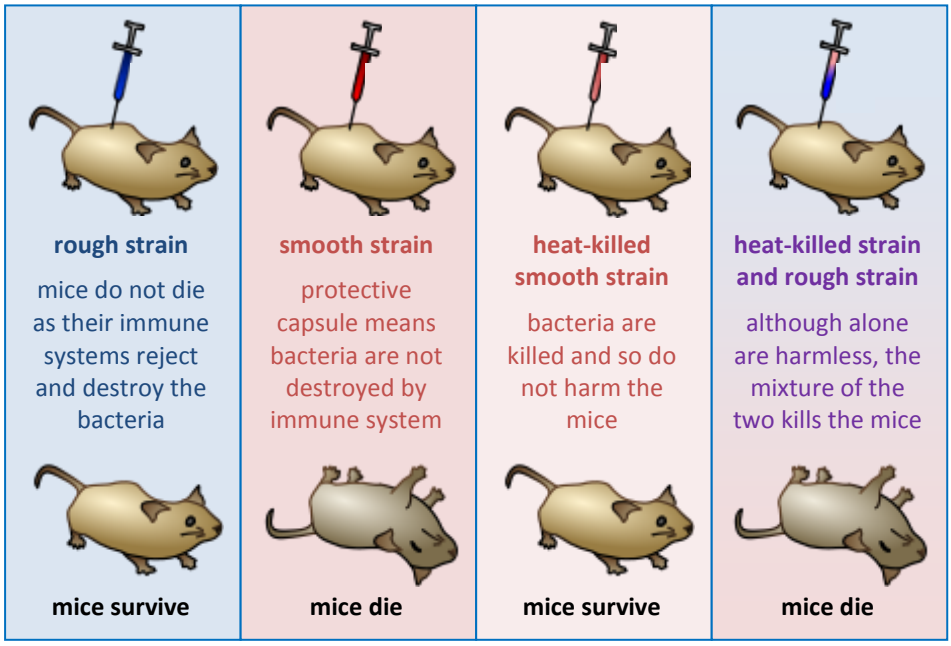


d The cells move apart and the plasmid in each forms a circle

Evidence for conjugation first came about in the studies of Frederick Griffith, in 1928 before the importance of the DNA molecule was established later by Watson and Crick. In the **Griffith experiment**, mice were infected with two different strains of bacteria. The four conditions of his experiment were:

- firstly, mice were infected with the **S-strain** of the bacteria (smooth strain) which kills the mice quickly upon infection, as this strain develops a polysaccharide coating which protects it from the host's immune system
- secondly, different mice were infected with the **R-strain** of the bacteria (rough strain), which did not kill the mice as it does not develop the protective coating and so is destroyed by the mice's immune systems
- next, Griffith infected some mice with a heat killed S-strain, and they did not die, as even though this strain was harmful – the bacteria had been killed by heating them to high temperatures
- but then other mice, who had been infected by a mixture of the R-strain and the heat-killed S-strain (neither of which alone killed the mice) were killed by this mixture

The results are summarised in the diagram to the right. Since practically nothing at this time was known about DNA itself, let alone bacterial conjugation, the results stunned Griffith.



A post-mortem examination on the dead mice killed by the mixture of the heat-killed S-strain and the rough strain revealed that the mice had living bacteria of both the R-strain and the S-strain, which was even more a surprising observation, given the mice had been infected with a dead strain of the S-strain, so how was it that a live version of the S-strain was present in these mice?

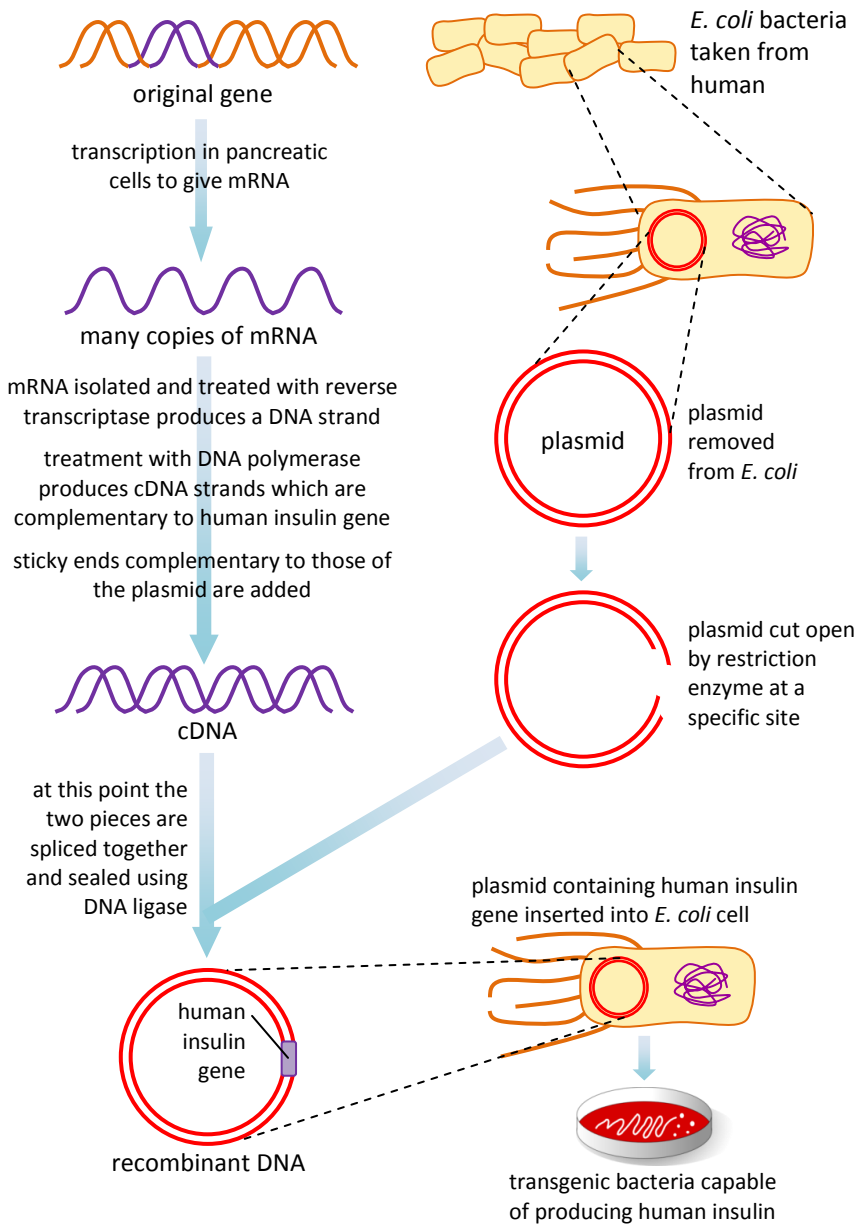
Little did Griffith know, that this was actually the first experiment to show bacterial conjugation. It was the first to show how bacteria can take up DNA from their environment, and it was confirmed that this is what the R-strain could do. In this case, the R-strain had taken up some DNA which remained from the dead S-strain, which coded for the production of the protective capsule, which allowed the R-strain to be toxic to the mice. The bacteria had *transformed*.

Engineering case study: human insulin

Those who suffer from Type I diabetes are unable to manufacture the hormone insulin, and prior to the 1980s insulin was extracted from the pancreatic tissue of slaughtered pigs for clinical use – but this is not exactly the same as human insulin, so this was an inefficient and expensive method. Since the sequencing of the protein hormone insulin by Frederick Sanger (which earned him his first Nobel prize – the other for developing Sanger sequencing) it has been possible to use bacterial genetic engineering to produce insulin much more cheaply and quickly, using the human insulin gene.

Scientists focused on finding the mRNA strand coding for the insulin gene, and once it was located, the enzyme known as **reverse transcriptase** was used to synthesise a complementary DNA strand, which is single-stranded. Once this has been isolated, free nucleotides and **DNA polymerase** are added to the insulin gene in order to make that single-stranded molecule double-stranded as the enzyme builds a complementary second strand, producing a copy of the original gene, called a **cDNA** gene.

Plasmids from the bacterium *E. coli* are used in this process: they are cut open at specific points using *restriction enzymes*, and then the cDNA (which has unpaired nucleotides on either end, called *sticky ends*, allowing for annealing) is mixed with the open plasmids and the *DNA ligase* enzyme. Some of the plasmids will simply reseal themselves using DNA ligase, but some will take on the insulin gene, becoming recombinant plasmids, which can then be mixed with bacteria so that they take up the recombinant plasmids.



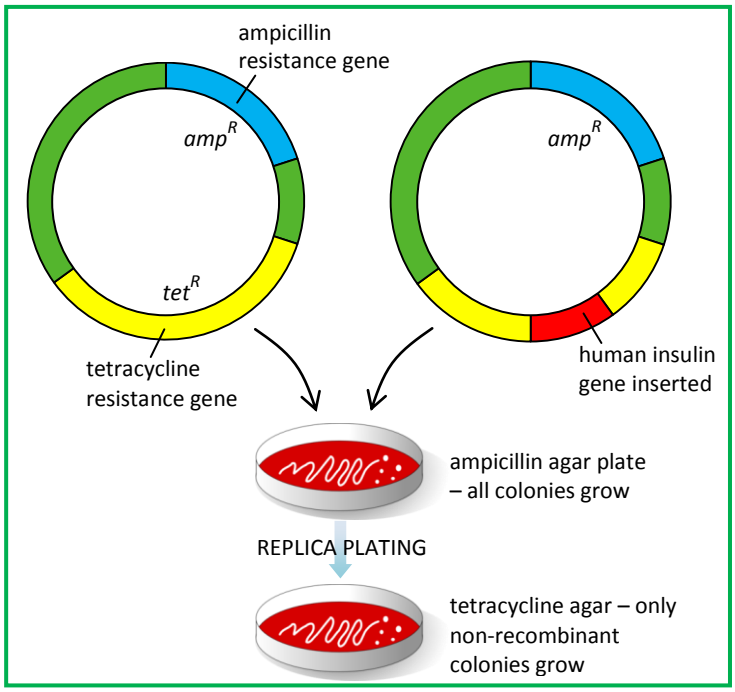
Bacteria can then be grown on an agar plate, and transformed bacteria will be capable of producing human insulin. If is important to remember that there are three possible types of colony that can be grown through this method: some bacteria won't take up a plasmid at all; some bacteria will take up the plasmids which simply shut themselves; and some bacteria will take up the plasmids with the insulin gene – this final type is described as the *transformed* bacteria and is the one we want to culture.

It is impossible to tell which type of colony has been produce simply by looking at the resultant bacteria, and so the transformed bacteria need to be identified. This process uses plasmid vectors with **genetic markers**.

The starting plasmids in the process are used because they carry genes that make them resistant to two different antibiotics (**ampicillin** and **tetracycline**), and these resistance genes are known as the *genetic markers*. The plasmids are cut by a restriction enzyme that has its target site in the middle of the gene coding for tetracycline resistance, so that if the required gene (in this case, insulin gene) is taken up, the tetracycline gene will be broken and so the bacterium would not have resistance to it – although the ampicillin resistance gene would remain unaffected.

A process known as **replica plating** then occurs:

- first of all, all the bacteria are grown on a standard agar nutrient plate so all colonies grow
- colonies are then transferred to an ampicillin agar plate so that only those bacterial cells which have taken up a plasmid (either with or without the insulin gene) will grow
- some cells from these colonies are then transferred to a tetracycline agar dish so that only those which have taken up the plasmid *without* insulin will grow
- by keeping track of our colonies, we are able to say that those which grew on the ampicillin agar plate but not the tetracycline plate must have taken up the human insulin gene (breaking the gene for tetracycline), so those desirable colonies can be identified and grown on a large scale and harvested



Engineering case study: Golden Rice™

A deficiency in **vitamin A** can have serious effects, such as leading to blindness, and there are an estimated 2 million deaths annually worldwide associated with a lack of vitamin A in the diet. Malnutrition is most common in the less economically-developed countries, which puts those people at serious risk.

Vitamin A (**retinol**) in the diet only comes from animal sources, but those who are vegetarian or don't have access to meat get their vitamin A from the intake of **beta-carotene**, a precursor to retinol, which is converted into vitamin A in the human gut. Vitamin A is fat-soluble, so the diet must contain some lipids in order for vitamin A to be taken up.

Rice plants contain the genes coding for beta-carotene, and this molecule is a photosynthetic pigment, and so is required in the green parts of the plant – unfortunately the genes for beta-carotene production are switched off in the grain part of the plant: the bit we eat. Scientists just over a decade ago worked to genetically engineer rice plants in order to get beta-carotene to accumulate in the grain part of the plant (the **endosperm**) that we eat. The product is **Golden Rice**.

Most of the enzymes to catalyse the metabolic pathway synthesising beta-carotene were found to actually be present in the endosperm. The researchers saw that inserting two genes (for *phytoene synthetase* and *Crt-1 enzyme*) near a specific **promoter region** switched on the genes during the endosperm development, so they were expressed.

Golden Rice is said therefore to be **biofortified**, containing higher than regular concentrations of beta-carotene. The usefulness of the product in treating and preventing vitamin A deficiencies is questioned, as many believe you would have to eat much more rice than normal for any effect, developed countries have failed to find an alternative way of tackling the issue.

The low beta-carotene content in Golden Rice has fuelled the criticisms of the project, with many arguing that it is less of a humanitarian approach than a positive public relations approach. Organisations such as Greenpeace have criticised Golden Rice with their opinions that genetic modification of crops will reduce biodiversity and that the safety to humans of such genetically-modified foodstuffs is unknown. They also believe that the production of Golden Rice is used as a public relations campaign to promote the use of genetic modification.

After food safety investigations have taken place, it is expected that full field trials, growing Golden Rice in a natural environment by mass will begin by 2012.