TOPIC: GENETIC MANIPULATION

Key Knowledge:

- the use of recombinant plasmids as vectors to transform bacterial cells as demonstrated by the production of human insulin
- the function of CRISPR-Cas9 in bacteria and the application of this function in editing a genome
- the use of genetically modified and transgenic organisms in agriculture to increase crop productivity and to provide resistance to disease.
- potential uses and applications of CRISPR-Cas9 to improve photosynthetic efficiencies or crop yields

GENETIC MODIFICATION

Genetic modification describes the alteration of a genome using genetic engineering technologies. This is typically achieved by the addition or silencing of a gene such that a function is either gained or lost. To be identified as a genetically modified organism (GMO), the change should be heritable. **Transgenics** is a type of genetic modification where DNA is introduced from an unrelated species. Hence not every GMO will be a transgenic organism. Genetic modification can be realised by creating entirely new organisms using gene cloning techniques, or by altering existing organisms with techniques such as CRISPR-Cas9 (gene editing).

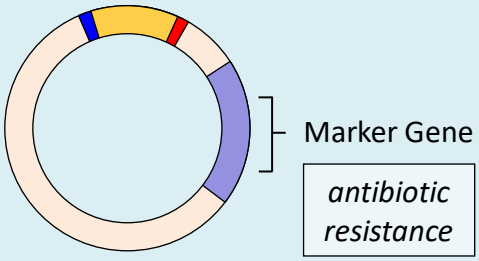
GENE CLONING

Gene cloning refers to the process of isolating a DNA sequence of interest for the purpose of making many copies of it. The gene of interest is isolated (via PCR) and then inserted into a plasmid vector (via digestion and ligation). The plasmid vectors are capable of autonomous self-replication within a host cell, ensuring the sequence is cloned. Recombinant vectors can be used to create genetically modified organisms (GMOs) which in turn can be used to produce large quantities of therapeutic proteins (**biopharming**).

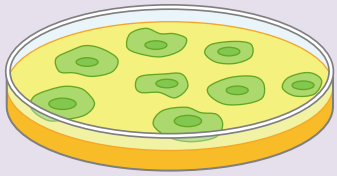
The process of gene cloning involves a number of steps:

- Isolate and amplify the gene of interest (and plasmid vector) with the polymerase chain reaction (PCR)
- If the gene is to be incorporated into a bacterial cell, a cDNA copy is required (reverse transcription)
- Digest gene and plasmid vector with the same specific restriction enzymes (blunt ends **or** sticky ends)
- Create a recombinant plasmid by ligating the gene of interest into the plasmid vector (with DNA ligase)
- Separate recombinant plasmids from normal plasmids (with no gene of interest) via gel electrophoresis
- Introduce recombinant plasmid into target cell via a vector delivery method (e.g. electroporation)
- Grow cells on antibiotic media to select modified cells (only cells with plasmid are antibiotic resistant)

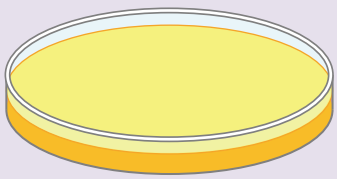
Recombinant DNA construct



Testing for Successful Gene Transfer



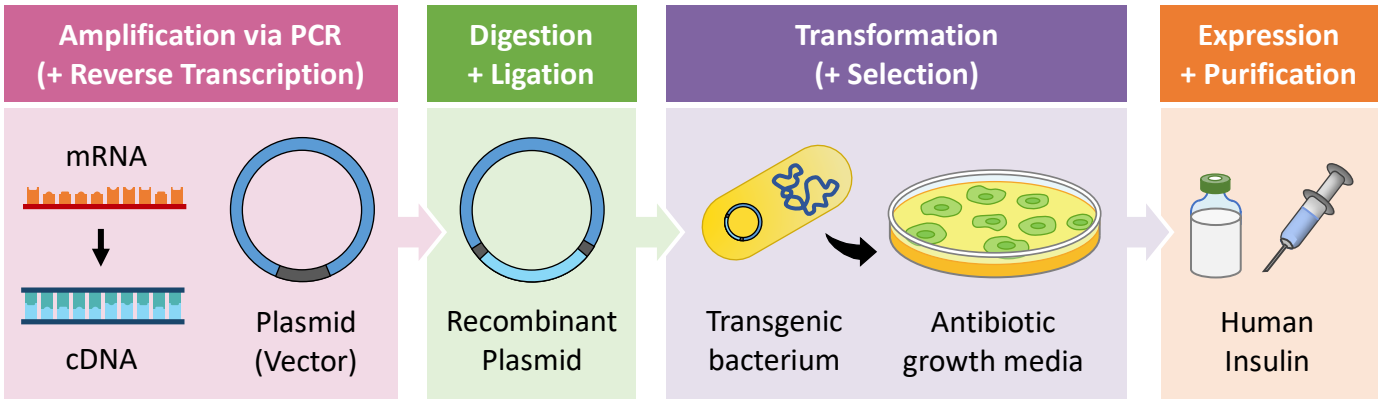
Cells with plasmid will grow in the presence of antibiotic



Cells without plasmid are killed by antibiotic

INSULIN PRODUCTION

As the genetic code is **universal**, genetic information is transferrable between species. The human insulin gene has been inserted into bacterial cells which can rapidly divide and hence produce large quantities of the protein for therapeutic use (i.e. in the treatment of diabetes). A cDNA copy of the gene is synthesised from an mRNA template using the enzyme **reverse transcriptase**. This ensures that the human insulin gene does not contain introns (bacteria cannot splice). The gene is inserted into a plasmid vector and is then transformed into the bacteria cell via a variety of methods – including electroporation (delivering an electrical current to permeabilise a cell), microinjection (using a micropipette to inject the vector), heat shocking (permeabilising the cell via the application of heat) and lipofection (transferring in a liposome).



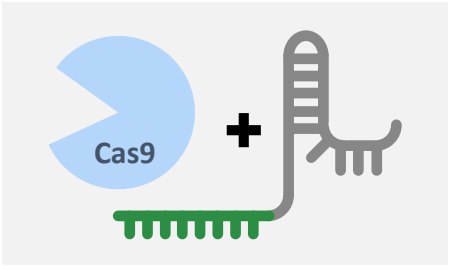
GENE EDITING

The CRISPR-Cas9 system is used by bacterial cells as a method of protection against viral pathogens. When infected, a bacterium will excise short viral sequences and paste them into palindromic sequences within its genome to form **CRISPR loci** (clustered regularly interspaced short palindromic repeats). The CRISPR sequence acts as a memory bank for the viral infection and is transcribed into a **guide RNA** strand (gRNA) that interacts with CRISPR-associated nuclease (**Cas9**). The gRNA locates and binds to any complementary viral DNA, enabling the Cas nuclease to destroy the viral sequence (and hence prevent the viral infection).

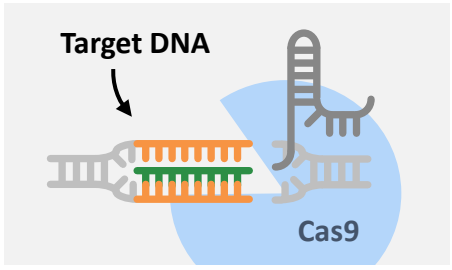
The CRISPR-Cas9 system has been modified by scientists to selectively remove any targeted sequence:

- A synthetically derived guide RNA molecule is developed that is complementary to a target sequence
- The guide RNA will associate with Cas nuclease and promote the excision of the target sequence
- With the excision of a target sequence, another sequence can be integrated in its place (gene editing)

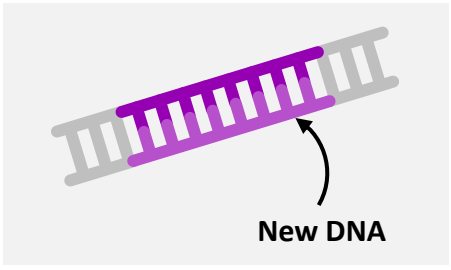
The CRISPR-Cas9 system has the potential to treat a range of medical conditions with a genetic component including cancers, hepatitis or even high cholesterol. One current limitation with this system is that gRNA typically consists of a short sequence (roughly 20 bases), which could exist in other locations within the genome and cause excisions at unintended locations (leading to unknown health consequences).



Guide RNA added to cell along with Cas9 nuclease



Guide RNA binds to a target sequence which is excised



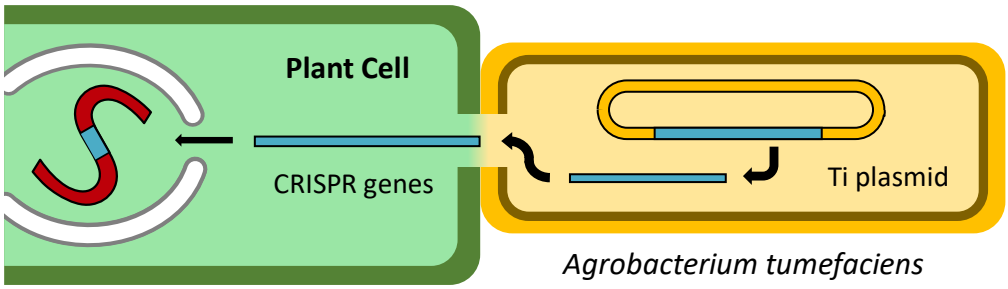
Excised segment is replaced by new DNA (gene editing)

TRANSGENICS

Transgenic organisms contain DNA originating from another species (and introduced via artificial means). This is possible because the genetic code is universal. The foreign genes can be introduced either via gene cloning (i.e. incorporation of a recombinant plasmid) or gene editing (i.e. utilising the CRISPR-Cas9 system). The advantage of gene editing with CRISPR-Cas9 is that in addition to being able to introduce new genes into an organism (gain of function), it can also be used to remove pre-existing genes (loss of function).

Gene editing of crops will commonly involve the use of *Agrobacterium tumefaciens* as a delivery vehicle. This bacterium possesses the **Ti plasmid** which will integrate its own DNA into the plant cell genome. By incorporating the genes for the Cas9 protein and the gRNA sequence into the Ti plasmid, the CRISPR-Cas9 system can be effectively introduced into plant tissue.

Transgenic crops have been used in agriculture for a variety of purposes, such as conferring tolerance to environmental conditions (e.g. drought, salt tolerance, colder temperatures) in order to increase the overall productivity of the crop. Other crops have incorporated genes encoding for vitamins in order to improve the nutritional content of food (e.g. Golden rice produces higher levels of Vitamin A). Scientists have also introduced genes from C_4 plants to improve photorespiration efficiencies in C_3 plants. Finally, crops have been modified to produce natural insecticides (e.g. Bt corn), reducing the use of pesticides.



Agrobacterium tumefaciens

A **Ti plasmid** within *A. tumefaciens* can insert recombinant DNA (CRISPR genes) into a plant genome

IMPLICATIONS AND ISSUES

There are biological, environmental, social and economic issues associated with transgenic crop use:

BIOLOGICAL

GM crops can be used to improve nutritional standards in humans by incorporating genes for particular proteins, vitamins or vaccines

The inclusion or removal of certain genes could trigger adverse health reactions (e.g. allergies)

ENVIRONMENTAL

GM crops will potentially reduce the need for deforestation (can farm a wider area) and reduce the use of pesticides (via pest-resistant crops)

GM crops could either cross-pollinate or directly compete with native flora to reduce biodiversity

SOCIAL

Patent protections may allow biotech companies to restrict the use of seeds and force farmers to pay high prices leading to equity of access issues

Different governments may have conflicting regulatory standards concerning safe usage

ECONOMIC

GM crops can have increased disease resistance, grow in a wider range of conditions and produce greater yields – increasing the financial benefits

GM crops may include genes which slow the rate of spoilage, leading to longer overall shelf lives