BIOTECHNOLOGY

Content Statements:

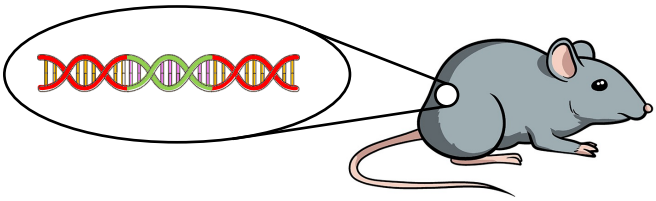
D1.3.8 Gene knockout as a technique for investigating the function of a gene by changing it to make it inoperative

D1.3.9 Use of the CRISPR sequences and the enzyme Cas9 in gene editing

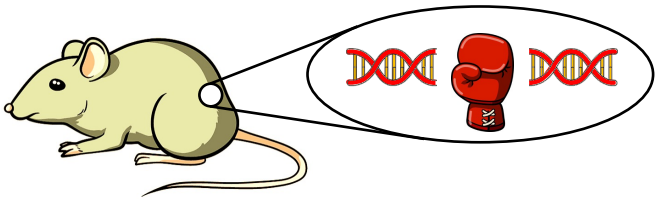
D1.3.10 Hypotheses to account for conserved or highly conserved sequences in genes

GENE KNOCKOUTS

One way that scientists can investigate the function of a gene is by making it inoperative within an organism (called a gene '**knockout**'). The knockout can then be compared against a wild-type (control) to deduce the function of the gene. Scientists can either knockout a gene across an entire organism or target the knockout to a particular tissue or organ. There are various approaches that can be used to silence a gene – including the Cre-LoxP system, RNA interference or CRISPR-Cas9 editing. A library of knockout organisms is available for some species for use as models in research. Conditions that are currently being investigated via the use of knockout organisms include obesity, diabetes, cancer, ageing and addiction. Mice are commonly used as model organisms due to a high degree of genetic similarity to humans – but other organisms can be used.



Normal Mouse (Target Gene Expressed)



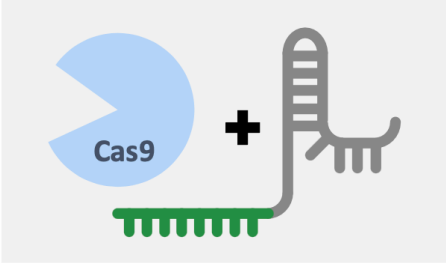
Knockout Mouse (Target Gene Silenced)

CRISPR-CAS9 SYSTEM

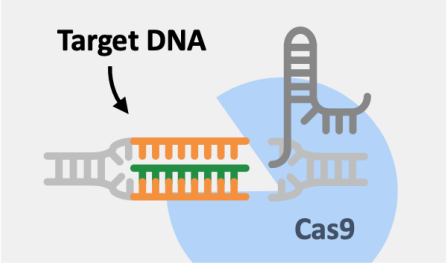
The CRISPR-Cas9 system acts naturally in bacteria to provide immunity against viral infections by removing viral genes that have been integrated into the host genome. When a virus infects a bacterial cell, snippets of viral DNA are pasted into the bacterial genome to form a **CRISPR** locus. These CRISPR sequences act as a genetic memory bank and are transcribed into guide RNA strands (**gRNA**) that bind to a CRISPR-associated nuclease (**Cas9**). The gRNA-Cas9 complex drifts throughout the cell until the gRNA locates and binds to any complementary viral DNA. This enables the Cas9 enzyme to excise the viral sequence from the genome. Once the viral DNA has been excised, DNA repair enzymes function to rejoin the DNA fragments and reform a continuous strand. Because the viral DNA has been removed, the CRISPR-Cas9 system can be said to have functioned as a gene knockout mechanism. Humans do not possess CRISPR sequences or the Cas nucleases required to knock out genes, although gene cloning techniques are being utilised to implement this system.

GENE EDITING

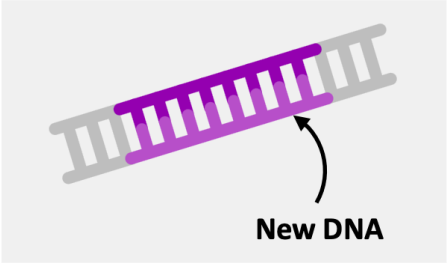
The CRISPR-Cas9 system has been modified by scientists to potentially enable the removal of any selected sequence, allowing for the precise gene editing of a cell or organism. The Cas9 protein is complexed with a **synthetically derived gRNA** molecule that is complementary to a target sequence. This gRNA will bind to the target sequence, prompting its excision by the Cas nuclease (i.e. gene knockout). Following the removal of the target sequence, another sequence of DNA can be integrated in its place. This allows the scientists to effectively edit gene sequences – either removing or introducing mutations to treat a variety of conditions.



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)

GENE EDITING APPLICATIONS

Gene editing via the CRISPR-Cas9 system has been widely used in agriculture to improve food production:

- Certain metabolic pathways have been enhanced to improve nutritional content (e.g. higher starch)
- Plant absorption spectra have been modified to increase photosynthetic efficiencies (more pigments)
- Higher tolerances to pathogens or environmental stresses (cold, drought, salinity) have been achieved
- Resistance to herbicides have been incorporated to reduce the costs associated with spraying weeds

CONSERVED SEQUENCES

Certain DNA sequences may show minimal levels of change over long periods of time and can be used to establish evolutionary pathways. A sequence that is identical (or highly similar) across all members of a species or a group of species is called a **conserved** sequence, while a sequence that remains identical (or highly similar) over long periods of evolution is called a **highly conserved** sequence. Because these DNA sequences don't change much over time, they can be used to demonstrate any phylogenetic relationships. For example, the presence of 355 highly conserved genes in prokaryotic organisms was used as a basis for determining the characteristics of the last universal common ancestor (i.e. chemotrophic thermophile).

There are two hypotheses to account for the presence of conserved or highly conserved gene sequences:

1. The gene serves an **essential functional requirement** within the cell – The removal of an essential gene would prevent organism survival, meaning it is unlikely that any mutations to the conserved sequence would be maintained within a gene pool. Examples of essential genes would include those encoding enzymes involved in key metabolic processes, such as cell respiration, transcription, translation, etc.

2. The gene has a **slower rate of mutation** – Some sequences are more likely to mutate (e.g. methylated cytosines can deaminate to form thymine). Additionally, the position of a gene may affect the rate of mutation (e.g. the proximity of a gene locus to the telomere may influence mutation rate). Genes with higher expression levels also have more active DNA repair and proofreading mechanisms – resulting in fewer mutations remaining unchanged (which means the sequence is more likely to be conserved).