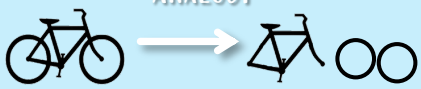
D1.3 Mutations & Gene Editing (HL)

GENE KNOCKOUT

Rendering a gene unusable to see the effect it has on an organism, and hence its function.

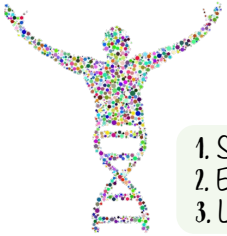
ANALOGY



By removing the wheels, we can see what their function was by analyzing the leftover "frame".

Human Genome Project

A project that aimed to find the base sequence (order) of our genome. → Gene knockout is used to help understand the purpose of each gene.



Test on humans

1. Expensive
2. Unethical
3. Dangerous

Knockout organisms

1. Share many of the same genes
2. Easy to maintain and control
3. Life cycle is much shorter

Test on Mice OR model organism*

* One that is used in place of another organism for ethical and practical purposes.

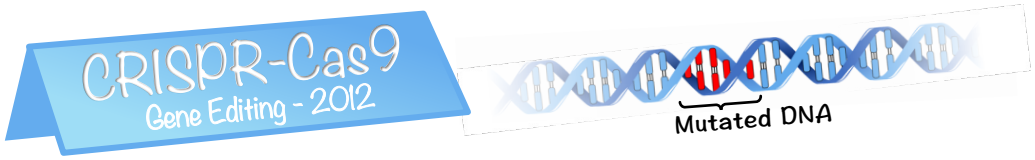
Purposes of gene knockout:

1 Studying gene function
By knocking out genes, we can figure out what their purpose is, helping us understand how we function

2 Modeling human disease
By replicating disease in knockout mice, it helps researchers understand disease mechanism and test potential treatments.

3 Developmental biology
Knocking out genes involved in embryonic development can help scientists understand birth defect and developmental disorders.

A technique called **CRISPR-Cas9** was developed which allows for genes to be edited; whereby mutated genes can be removed and/or replaced with an unmutated/new gene:

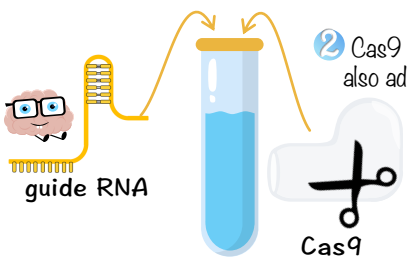


Guide RNA (gRNA)

Cas9

PAM sequence

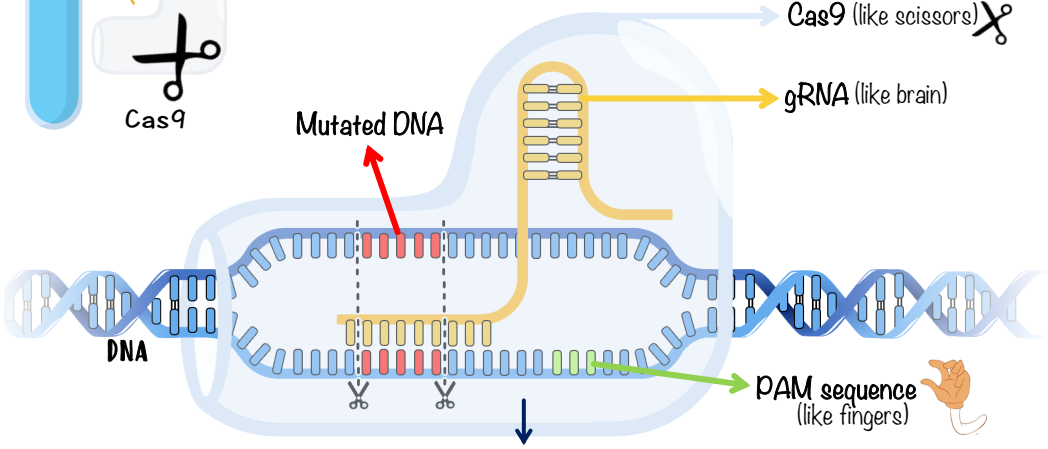
1. Mutated DNA is added to a guide DNA which was created to match the mutated DNA.



2. Cas9 (scissors) are also added to the mix.

3. The **GUIDE RNA** (gRNA) and the **CAS9 PROTEIN** attach to each other and this mix is injected into cells.

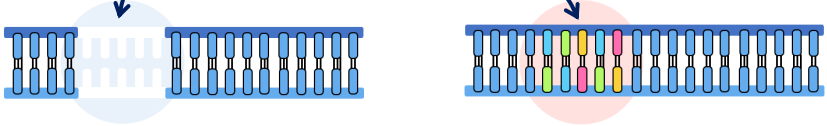
4. The guide RNA identifies the mutated DNA segment, and Cas9 uses a **PAM SEQUENCE** (protospacer adjacent motif) found near the mutated DNA to anchor to the DNA.



5. Cas9 cleaves the DNA double strand, removing the mutated segment of DNA.

Unmutated gene/ New gene

6. Mutated gene is removed or attempts at repair lead to errors leading to the inactivation or silencing of the gene.



7. Mutated gene can otherwise be replaced with a functional (not mutated) version of the gene OR a new gene.

D1.3 Mutations & Gene Editing (HL)

Advantages of Gene Editing

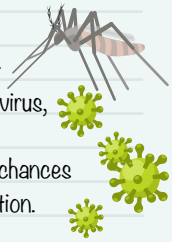
1. Delete harmful gene

Useful in single gene disorders - Huntington's or sickle cell disease
Even delete a full chromosome – Down syndrome

2. Optimize / Amplify function

Agriculture – more food and more resistance, make food more nourishing or livestock more productive or resistant to disease.
Example - One CRISPR-Cas9 project, produced tomato plants that yielded twice as much as non-modified plants.

3. Disease transmission



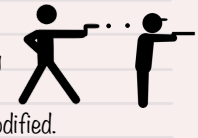
Human health – modifying mosquitoes so that they can no longer transmit the dengue fever virus, malaria parasite or west nile virus.
Gene drive – a mechanism that increases the chances of a gene being passed on to the next generation.

Disadvantages of Gene Editing

1. Germ cell effects (future effect)

If CRISPR-Cas9 is used in somatic cells, only the individual's genome is modified.
If it is germ cells, the individual is modified and can pass on the modification to future generations.

2. Off target effects



Another concern with gene modification is off-target effects. This is when the editing technology accidentally changes a part of the genome that was not intended to be modified.

3. Time

Even when possible, in the laboratory, it doesn't mean it can be used yet: still needs testing. Rules of safety and testing need to followed.

Conserved and highly conserved sequences

Conserved sequences: genetic sequences found in DNA or RNA that show **MINIMAL** mutations over time (in a species or population). Genomic* & bioinformatics*.

Highly conserved: those that show **NO** or almost no changes.

* **Genomics** – science of gene sequencing * **Bioinformatics** - Using computer programs to analyze the enormous data sets.

What is the mechanism?

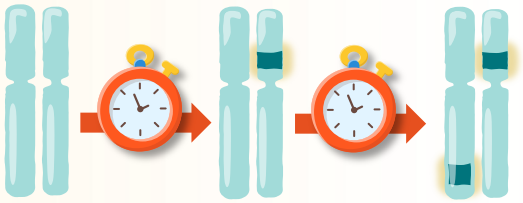
1. Slower mutation rates

Key sequences (high risk) of the genome has a **MORE ACTIVE** DNA repair system. Repair system is less active in low risk zones (non-coding regions, silences genes).

Zones with low mutation rate ≠ Less mutations

They are just corrected better than other zones [highly transcribed genes show lower mutation rates than less expressed ones.]

Mutation rate – refers to how many changes there are in a genetic sequence over time. It can be expressed as the number of base pairs changing in a single gene at each generation (or each cell division).

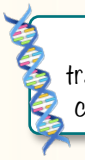


2. Functional requirements

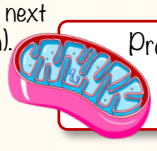
Sequences which are critically **REQUIRED** for the proper **FUNCTIONING** of the cell (see right) are more highly conserved.

Natural selection conserves such sequences by necessity (prevents mutated sequences from being passed on to the next generation as offspring wouldn't survive with the mutation).

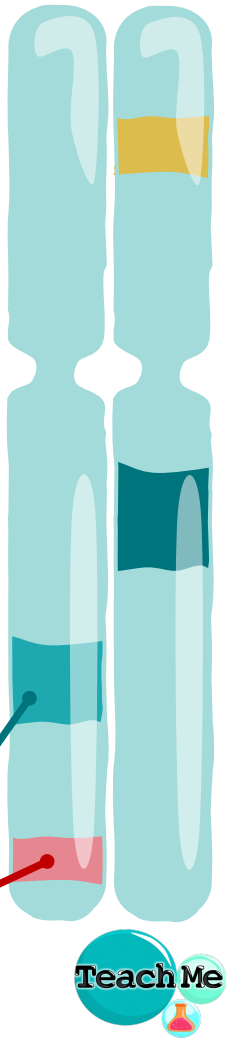
Purifying selection (or negative selection)
Phenomenon of eliminating harmful variation of genes.



Proteins used in DNA replication, transcription and translation: Helicase, non-coding RNA such as tRNA, and ribosomes.



Proteins used in ceullular respiration such as cytochrome c and ferredoxin etc.



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