GEN PHILIC

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Experiments in Genetics - 1

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Gene Drive Mosquitoes!!

Modifying pathogens genetically to counter disease spread

Mosquitoes are considered to be the most dangerous animals on earth. Through the transmission of deadly pathogens, they are believed to cause more than 700,000 deaths each year and are considered to be a major underlying cause of poverty in developing countries.
Over 15 years ago, a team at Imperial College London, lead by Professor Andrea Crisanti and Austin Burt was able to modify the genome of Anopheles mosquito for the first time. Just two years later it's full genome sequence was made publicly available, ushering in a new era in the fight against malaria. Austin Burt and Andrea Crisanti had been trying for eight years to commandeer the mosquito genome. In the back of their minds was an idea which could, if successful prevent malaria by spreading a gene to knock out mosquito populations such that they could no longer transmit the disease.
Finally in 2011, the two geneticists got back the DNA results they'd been anticipating: a gene-insert which they had introduced in the mosquito genome had radiated throughout the population, being housed in more than 85% of the insects' descendants.

This is when the first engineered GENE DRIVE came up. So a thought might pop up in your mind, what is this Gene Drive?
A Gene drive is a type of genetic engineering technique that aims to replace a natural gene with a new gene, which then gets passed on from generation to generation.

Thanks to CRISPR-Cas9, the widely used gene editing technology harnessed from bacteria, building a gene drive is not as harrowing a task as it once was for the researchers. With gene drive technology, "You can modify evolutionary trajectory. You can cause extinction." said Andrea Crisanti.

How exactly does this fancy thing work?

A Gene drive has three components --

1) The gene that you want to spread.
2) Cas9 enzyme which cuts DNA and
3) CRISPR, a programmable DNA sequence that identifies where the enzyme should cut.

In gene drive, the gene to be spread and the DNA sequence of CRISPR are inserted in the donor template of the animal's DNA . Now this animal is mated with it's opposite sex partner who doesn't possess this modification in their DNA. This results in an offspring with one copy of DNA from each parent (in this case, offspring will have a gene drive version from one parent and a normal version from the other parent).

During this type of mating, before the beginning of embryonic development, the CRISPR sequence which is present on the Gene Drive DNA gets activated and it recognises the natural gene on the opposite homologous chromosome. Furthermore, the DNA cutting enzyme, Cas9 cuts this natural copy and damages the chromosome. This incites the DNA repair machinery of the damaged cell to get switched on. This repair machinery the proceeds to use the gene drive chromosome as a template and repairs the damage. In this way, the gene duplicates itself, as a result of which, both the chromosomes come to own a copy of the gene drive (gene of interest).

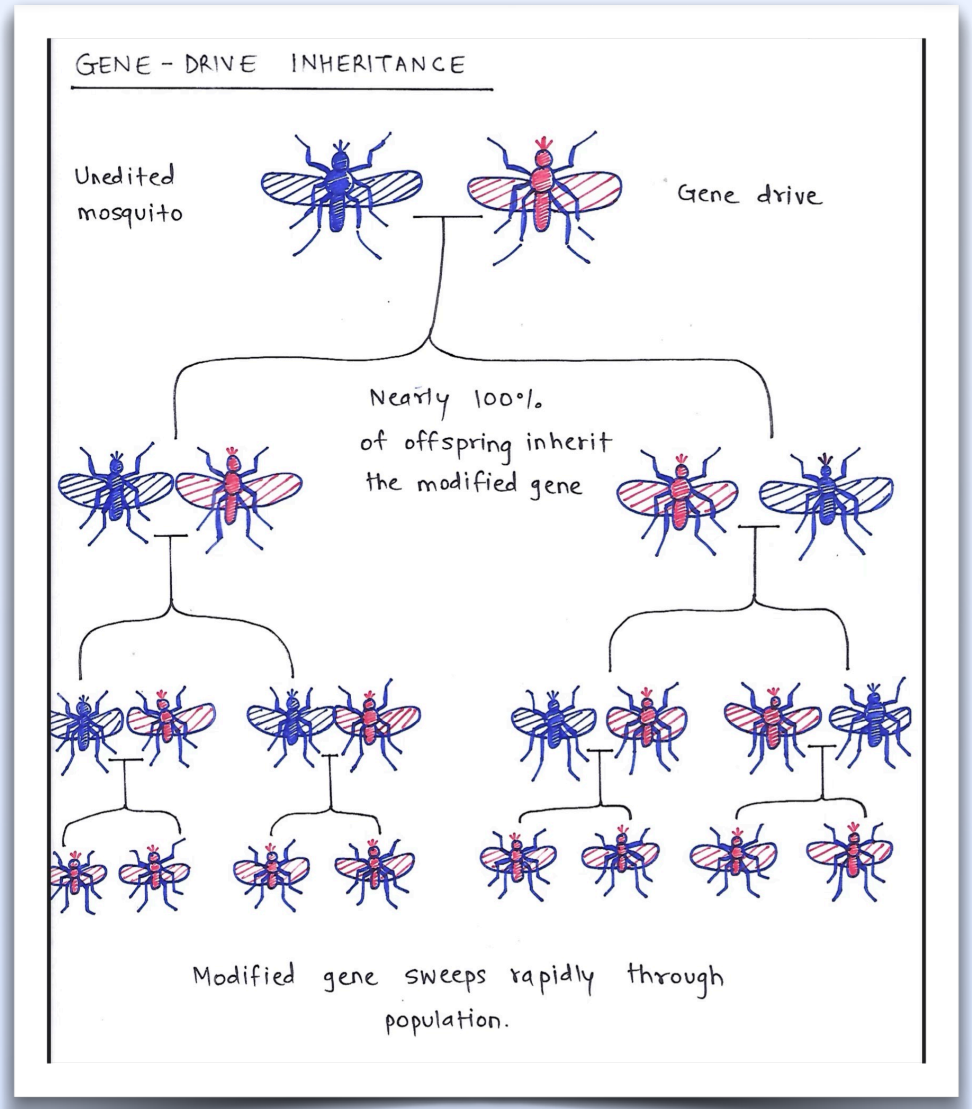

Illustration 1: Propagation of Genetic modifications in population

Illustration by : Aarya Kuvalekar
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These two copies can now be passed on to the next generations, wherein each generation will possess at least one copy of the gene of interest.
Following which, the process continues. Each time the drive is passed on, CRISPR cuts the

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natural version of the gene, cell repair machinery intervenes and one copy of the gene drive becomes two.

Using Genetic Engineering, scientists successfully created a strain of mosquitoes that is immune to the Malarial parasite by adding new antibody gene that specifically targets plasmodium. These mosquitoes will never spread Malaria.

Having said that, just changing genetic information is not enough as the edits would only be inherited by half the offspring. This is because most genes have two versions inside a diploid genome. Therefore, after two generations, at most only half of the offspring population would carry the engineered gene. And that would be but a drop in the ocean that is the population of mosquitoes!!!

Gene Drive's main purpose is to solve this problem. It forces a new gene or trait to traverse across the subsequent generations. This phenomenon holds the potential to eradicate the old gene almost completely. 99.5% of all engineered mosquitoes offspring will carry anti-malarial edit. If enough engineered mosquitoes are released to mate with normal mosquitoes, the malaria blocking gene would spread very rapidly.

In September 2018, Crisanti and his team performed an experiment with *Anopheles gambiae* mosquitoes which eliminated their population with an astounding efficiency of 100%.

In this experiment, they made a drive that disrupts a fertility gene known as "doublesex". With the drive in place, female mosquitoes could not bite and were not able to lay eggs. Within a span of 8–12 generations, none of the females produced eggs. The species could not mate and the population collapsed. Some researchers believe that this may be the approach that finally wipes out malaria. However, the research never stops and continues the endeavour to improve upon existing methods!! Now, the team is adapting the drive to try and cut not one but two loci on the doublesex gene.

> "I want to make sure that the likelihood of developing resistance is very, very remote before saying the technology is ready for the field,"
>
> — says Crisanti.

There are two broad approaches to malarial mosquitoes. The team at Imperial college is a part of an international group called 'Target Malaria' funded mostly by Bill and Melinda Gates Foundation. Bill Gates added his voice to support the use of gene drives to fight malaria in the recent speech at Malaria Summit London 2018 conference. He said, "I am very energized about the potential of gene drive, a method of self sustaining genetic change that can make mosquitoes infertile or prevent them from carrying the malarial parasite."

The drive focuses on spreading female infertility or to prevent females from being born so that the mosquito population shrinks. Additionally, there's also a team of researchers at the University of California who have been developing a gene drive that alters rather than shrinks the mosquito population. It spreads genes that make mosquitoes resistant to the malarial parasite so that they don't transmit it between humans. WHO has outlined certain steps that GM or genetically modified mosquitoes must go through before being deployed. These include screening tests, effects on biodiversity etc. Phase 1 is laboratory studies, but to find out if they can actually work, we have to test them outside the lab i.e. Field Testing (Phase 2). Mosquitoes will soon be ready and on their way to Phase 2.

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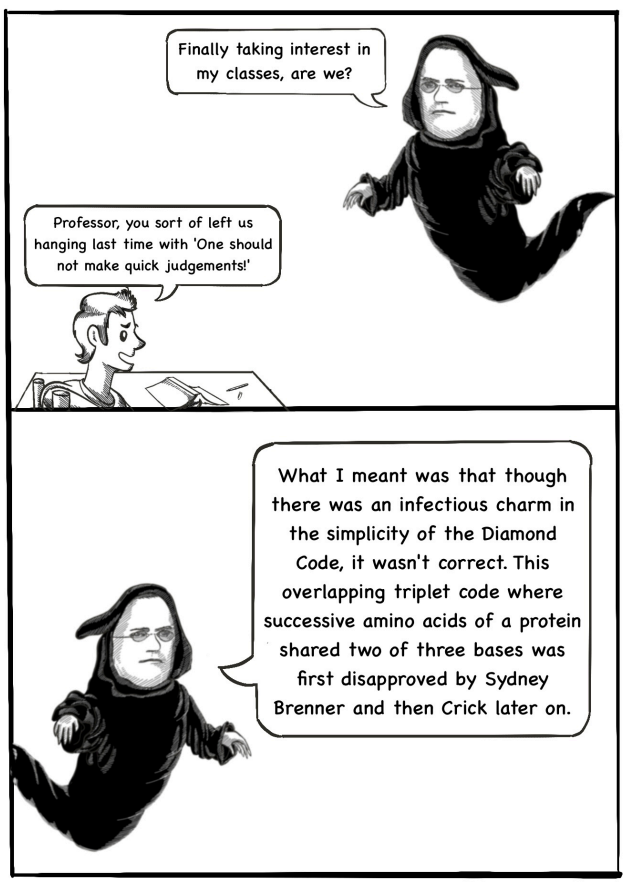
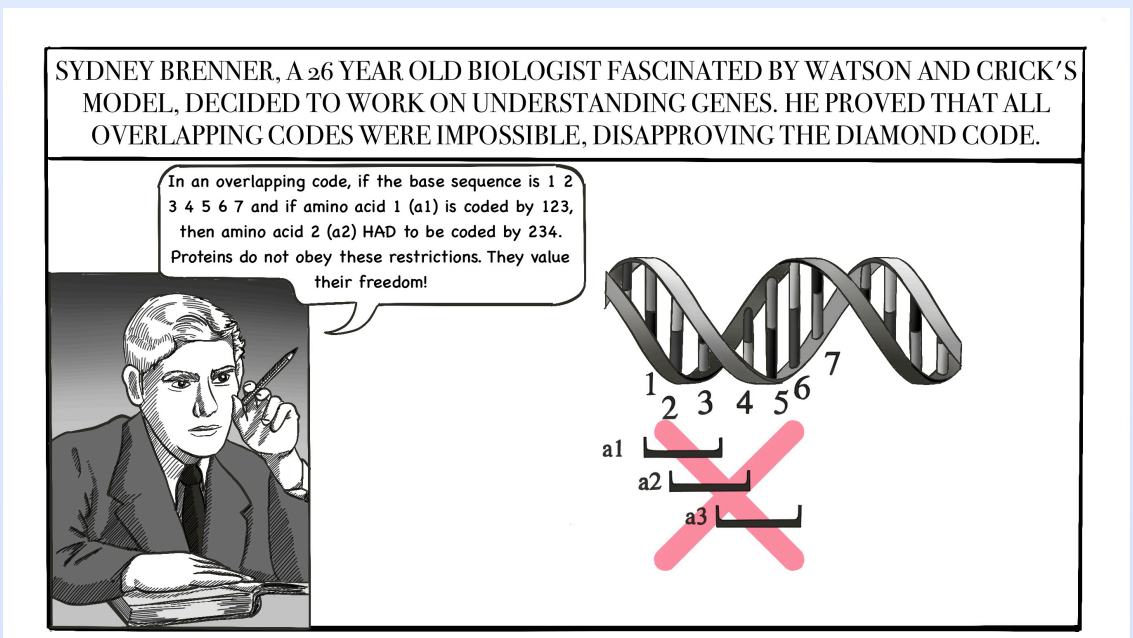
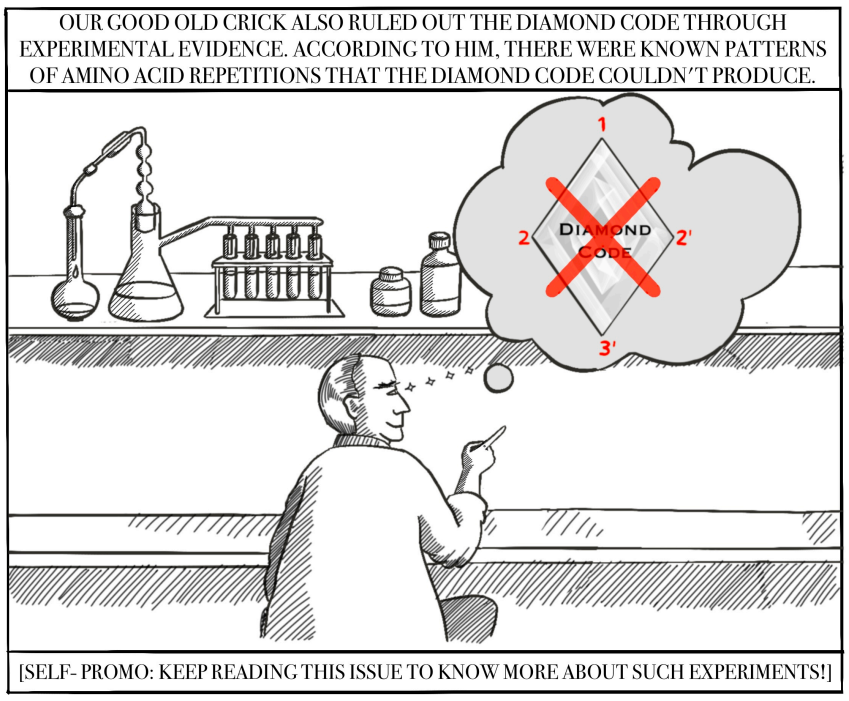
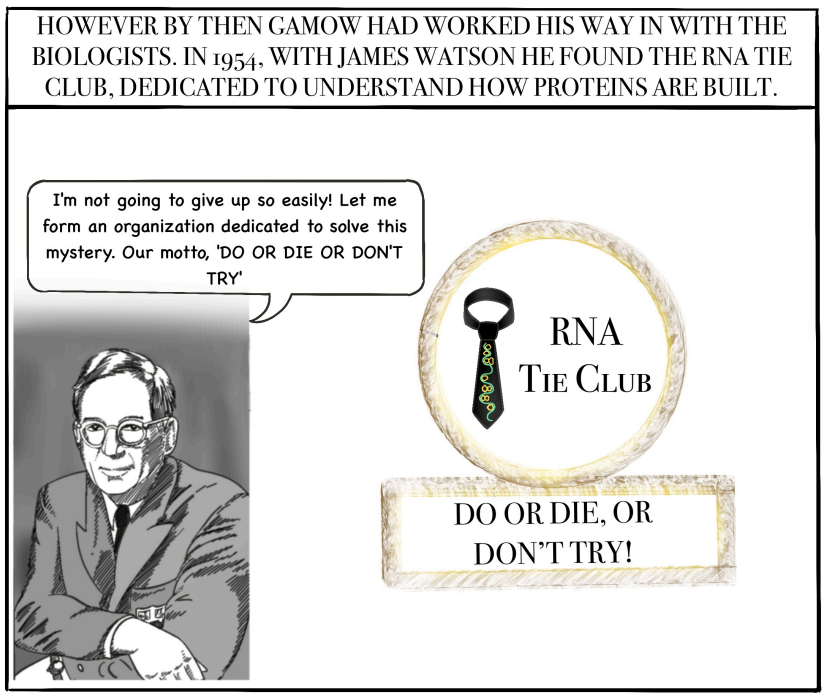
Back to the Basics with Friendly Ghost Grandpa Mendel!

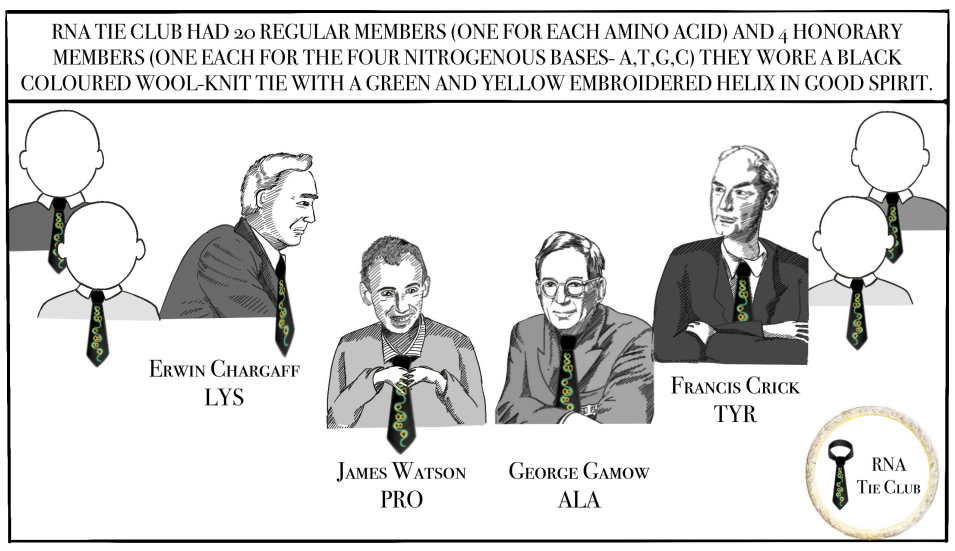
You know the legendary Gregor Mendel. You might not know the legends in the race to find messengers of life! Come, discover the story with Mendel himself, and see where the journey takes you! (Note : Read left to right)











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Solve our problems in other's bodies

Transgenic Animals as Models for Human Diseases

Transgenic animals are routinely used in the laboratory as models in biomedical research. They are important tools for researching human disease, being used to understand gene function in the context of disease susceptibility, progression and to determine responses to a therapeutic intervention. Transgenic animals are generated by adding foreign genetic information to the nucleus of embryonic cells, thereby inhibiting gene expression. This can be done by DNA microinjection, embryonic stem cell-mediated gene transfer and retrovirus-mediated gene transfer

Transgenic methodology serves as the link between molecular biology, introducing in vitro defined genetic modification and whole animal physiology, resulting alteration of body function. The pathology of all diseases, be they infectious, inherited or environmentally induced, is affected either directly or indirectly by an individual's genome. The recent sequencing of the human and mouse genomes has revealed that ninety-nine percent of the genes in these two genomes have direct counterparts in the two species. Mice breed rapidly, and methods of genetic modification are more effective, when compared with other mammals, hence mice are the preferred organisms for modelling the genetics of human disease.

Gene dysfunction is the root cause of all genetically determined disease processes. Not all gene dysfunctions are heritable as gene expression is also influenced by injury, infection, ageing, cancer, neural degeneration and neural regeneration. By asking how often mouse mutants reproduce the effect of mutations in the corresponding human gene, it is possible to assess the utility and relevance of disease models.

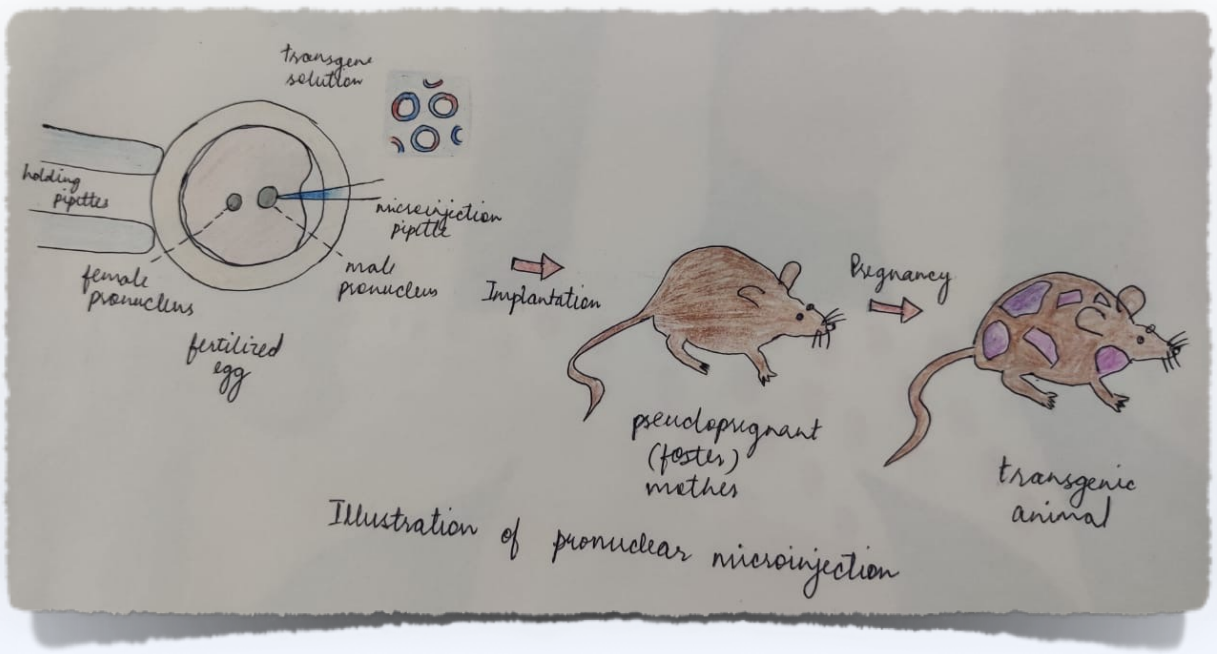
1. Diabetes: Mutations in the glucokinase gene in humans lead to a form of type II diabetes that manifests itself in the young, called maturity-onset diabetes of the young (MODY). These mice mutants provide a useful model of MODY and enable scientists to investigate the relationship between mutations in the glucokinase gene and the pathogenesis and severity of the disease.

2. Deafness: The shaker1 mouse mutant displays a profound hearing loss and was one of the first mouse mutants investigated as a model of human genetic deafness. Researchers identified the mouse gene underlying the shaker1 mutant and then located the corresponding gene in the human genome.

3.Psychiatric disorders: Scientists are exploring the role of the genes involved in certain inherited psychiatric disorders like autism, attention deficit-hyperactivity disorder, bipolar disorder, major depressive disorder and schizophrenia by examining their function in the transgenic mouse models, and their influence on other genes and neurotransmitter systems at the level of neurons and the brain.

4.Neurodegenerative disorders: Few neurodegenerative disorders, such as Parkinson's disease and Alzheimer's disease, are linked to single gene mutations. Reproducing the human form of these mutated genes in mice produces comparable pathologies to those in humans.

5.Cancer: Tumors arising in advanced GEMMs (Genetically engineered mouse models) closely mimic the histopathological and molecular features of their human counterparts, display genetic heterogeneity, and can spontaneously progress toward metastatic disease as GEMMs develop de novo tumors in a natural immune-proficient microenvironment.



Although an animal model cannot be considered as an exact replica of a human disease, scientists working in the field have found that there are often sufficient similarities to make informative comparisons. Even when animals do not present disease symptoms that are similar to those of humans, useful information may still be discovered regarding gene function.

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The Merry Game-te Corner

Code Decode

Fill in the blanks and unjumble the highlighted letters to decode the word of this issue.

i. A drive that disrupts a fertility gene known as: _ _ _ _ _ _ _ _ _

ii. The mouse mutant which displayed a profound hearing loss: _ _ _ _ _ _ _ _

iii. Crisanti and his team performed an experiment with Anopheles: _ _ _ _ _ _ _ _

iv. These mice mutants provide a useful model of MODY used for: _ _ _ _ _ _ _ _ _ _ _ _

Word of the Issue: _ _ _ _ _ _ _ _ _

Heredity Hunt

Find the words listed in the word search below:

MALARIA
TRANSGENIC
DIABETES
ANOPHELES
GENE DRIVE
MODY
GEMMS
TUMOR
CRISPR
CRISANTI

W	D	G	X	T	G	V	K	Q	A	U	W	H	T	S
G	A	R	F	Y	W	S	E	L	E	H	P	O	N	A
J	L	O	A	I	A	I	D	C	L	Z	K	L	W	Z
E	S	M	O	A	I	Q	U	I	O	F	I	P	B	A
O	V	U	A	V	T	E	S	N	A	L	Q	R	N	Z
R	G	T	J	L	N	V	O	E	S	B	O	A	V	X
G	P	U	Z	M	A	I	D	G	A	A	E	Y	U	E
D	J	S	T	S	S	R	S	S	J	J	C	T	V	B
S	W	O	I	U	I	D	I	N	E	H	E	R	E	J
H	M	P	N	R	R	E	T	A	K	Z	Y	B	M	S
O	Y	M	S	O	C	N	A	R	V	V	I	D	A	F
W	E	S	E	E	A	E	H	T	D	S	R	G	O	X
N	Y	J	K	G	J	G	I	Q	J	P	P	S	I	M

Gags By Gregor

Q. Why did the recessive gene decide to enter genetic therapy?
A. It wanted to learn how to express itself.

Created by : Divya Bhardwaj
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How did you find it? Any feedback? Suggestions?

We would like to know what you thought of this issue of Genophilic, any suggestions are welcome.

Our next issue will be on the topic 'From the Bookshelf'. Have you ever read a science fiction novel or story? We invite you to contribute to our next issue which will put forth book reviews for a few of the more interesting sci-fi publications, related to genetics.

Contact us on : editor.genophilic@gmail.com

References

Modifying pathogens genetically to counter disease spread

1. https://www.gatesnotes.com/Health/Most-Lethal-Animal-Mosquito-Week?WT.mc_id=MosquitoWeek2014_SharkWeek_tw&WT.tsrc=Twitter
2. https://www.nature.com/news/gene-drive-mosquitoes-engineered-to-fight-malaria-1.18858
3. https://www.nature.com/articles/d41586-019-02087-5
4. https://www.theatlantic.com/science/archive/2016/09/gene-drives/499574/

Transgenic Animals as Models for Human Diseases

1. https://www.nature.com/scitable/topicpage/the-use-of-animal-models-in-studying-855/#:~:text=Transgenic%20animals%20are%20generated%20by,into%20an%20organism's%20DNA.
2. https://www.whatisbiotechnology.org/index.php/science/summary/transgenic/transgenic-animals-have-genes-from-other-species-inserted
3. https://people.ucalgary.ca/~browder/transgenic.html
4. https://pubmed.ncbi.nlm.nih.gov/7795575/#:~:text=Transgenic%20animals%20have%20been%20used,processes%20on%20the%20cellular%20level.
5. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5286388/#:~:text=GEMMs%20of%20human%20cancer%20have,the%20potential%20of%20cancer%20immunotherapy.
6. https://uoqasim.edu.iq/e_Learning/lec_file/Microsoft%20Word%20-%20Animal%20Transgenesis,%20lecture%202.pdf