New inventions in the field of gene technology

Ms Vajirapani De Silva

When Rosalind Franklin took her x-refractory images of the DNA molecule over 60 years ago, she probably had no idea that her work was a mile stone in paving the way to "The unthinkable power to control evolution". As Jennifer Anne Doudna one of the leading ladies in modern-day gene technology, a major contributor in the discovery of CRISPR/Cas9 and programmable gene editing in living organisms, refers to the ability to delete , add or change the blue print of life in her book "A crack in creation", Doudna explains the possibilities of creating the unthinkable, considered to be impossible creations out of living cells. A brain child of Jennifer Doudna and Emmanuelle Charpentier gene editing is becoming a fast developing technology showing promise in applications in fundamental protein research for treatment of diseases including inherited genetic disorders and cancer. Like many other advanced technologies that were introduced in the past, this also is facing criticism due to cultural and ethical reasons. What is important is how we use such advanced techniques invented by man for the greater benefit of the planet, its inhabitants, and the preservation of earth for the future generations.

When the human genome project was completed in 2001, it was considered as an event equivalent to placing man on moon. There have been many leaps in life sciences since then with the advancement of gene mapping, research on genetic

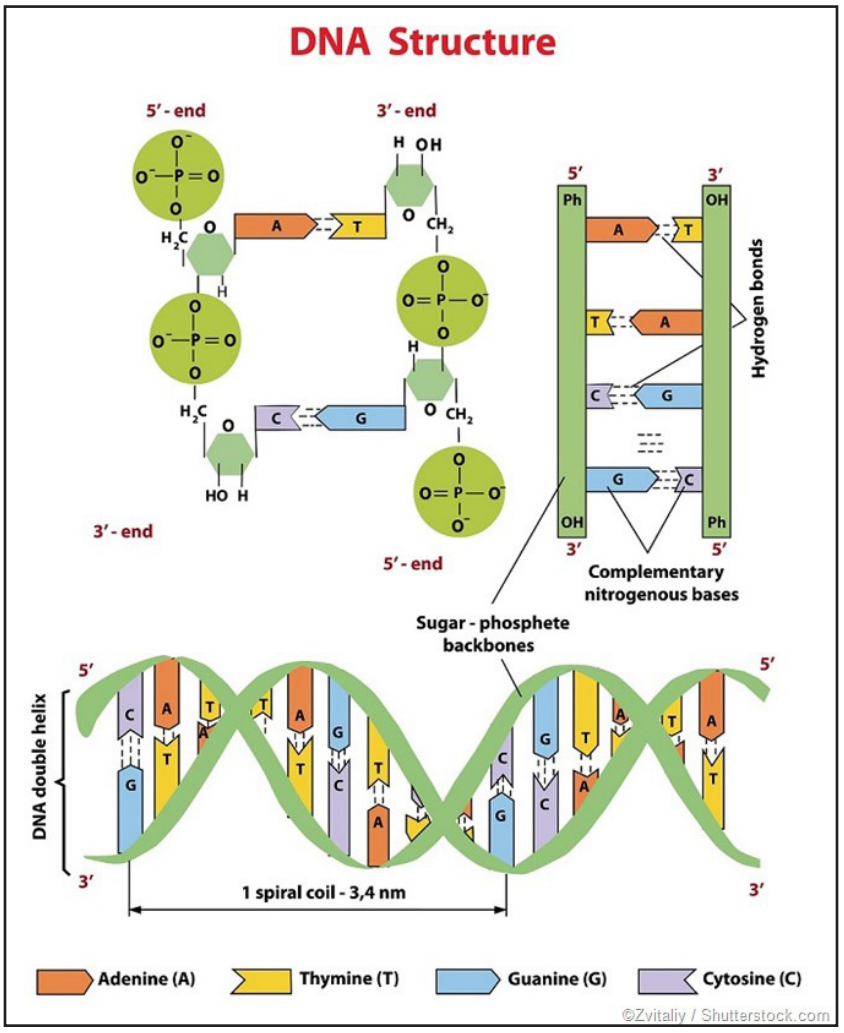


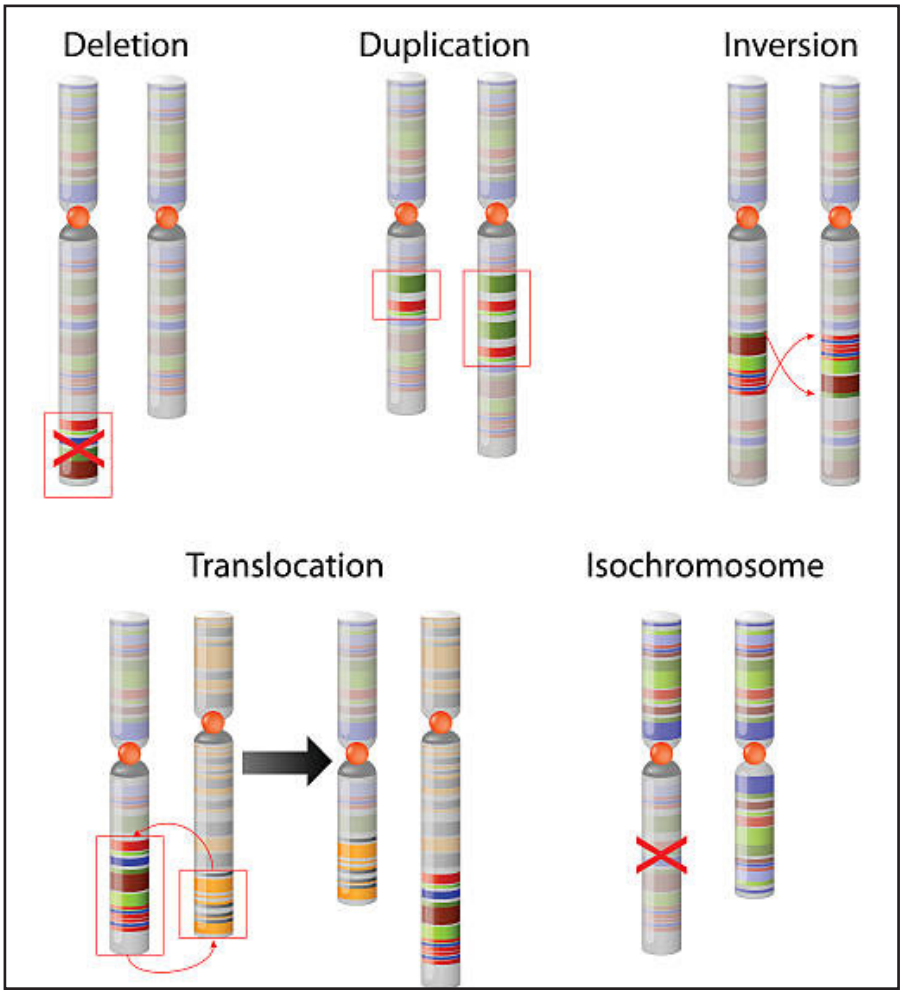
Fig : Mutations within chromosomes

disorders, gene therapy and gene editing. Though all these terms sound high-tech, they have been developed using simple principles of chemistry, and discovered by people who have dedicated themselves to research. Whatever the technique, DNA remains the blueprint of life with A, T, G and C making all the differences and similarities among the living organisms on earth.

Genetic disorders

Genetic disorders can be either inherited or non- inherited, and occurs due to random mutations in the genome. These mutations can be deletions, transfers , replacements , mixing of DNA between two different chromosomes , etc. Most of these disorders are considered to be incurable with the existing medical treatments. Genome mapping and sequencing have revealed valuable information on how these disorders occur, and consequently the control of gene expression studies can help in treating them. Epigenetics has become the major contributor to the study of control of gene expression.

Chromosomal Translocation

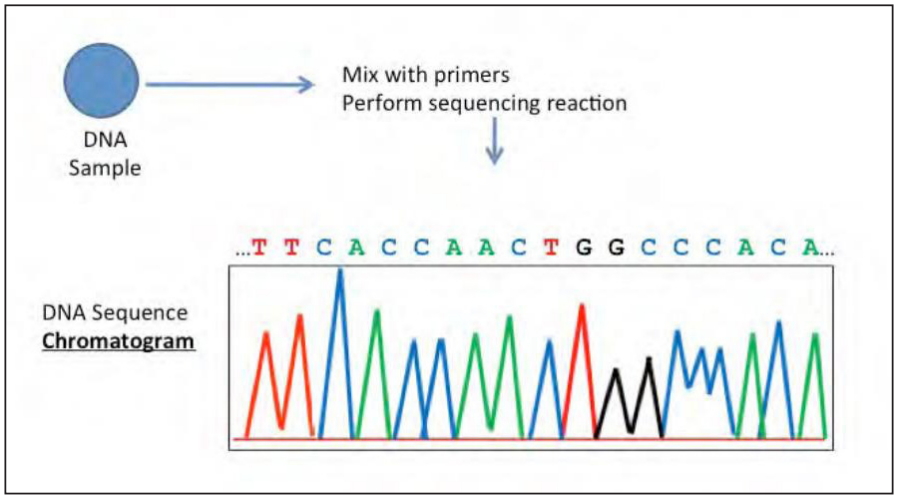


All these changes in the original sequence of DNA can lead to changes in the reading frames and hence producing abnormal proteins or destroying a gene that codes for a vital protein in the normal function of the organism. When occurred in reproductive tissues they carry the risk of being inherited by the offspring. Most of the time treatment has to be at the genetic level to re-correct these mutated DNA sequences. Gene therapy has shown promising results in the treatment of certain genetic disorders.

Genomics and Proteomics

It was known for a long time since the discovery of karyotyping, that just like an organism has a phenotype each of our chromosomes exhibit a phenotype when stained with specific staining dyes. Therefore with advances in microscopy and computer software, each chromosome can be identified with the numbers from 1 to 22, while X and Y have their own looks.But the differences within the similarities were found and revealed by the sequencing of the genome. Although the same location on a chromosome called locus codes for the same trait in the same species,the variation in that same trait comes from the different versions of the same location which are called alleles. Genomics and proteomics together can provide information on evolutionary advanced versions of these traits, hence allowing interfered selection of genes outside natural selection. Though there are many ethical aspects to be considered in manipulating genes as we want, the advantages of these interventions into nature can provide solutions to many modern day problems faced by our planet.

Overview of DNA Sequencing



Adenine (A) = Green
Thymine (T) = Red
Cytosine (C) = Blue
Guanine (G) = Black

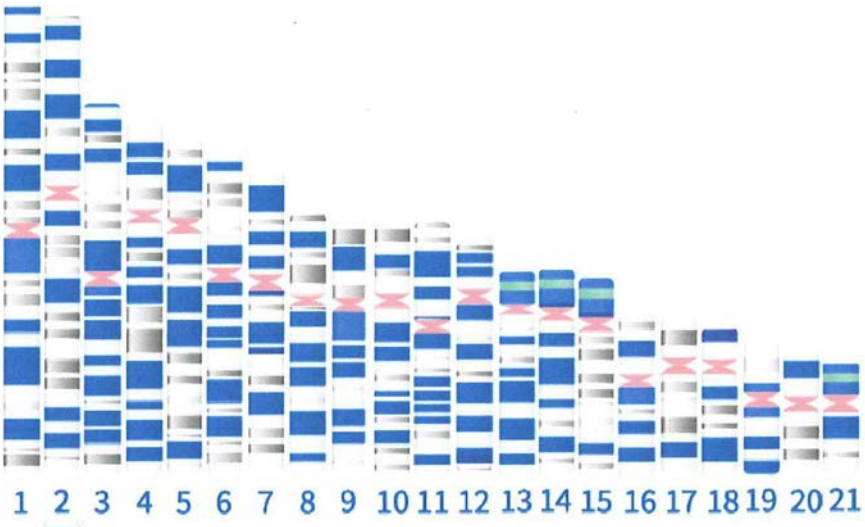
In the DNA chromatogram, each DNA base is represented as a peak of a different color. The DNA sequencing instrument "reads" the concentration measurement for each base and uses that data to determine the most likely identity for each base at each position. Sequencing instruments also produce text files showing the identity and order of all the bases (the DNA sequence).

Studying whole genomes and comparing genomes of different organisms fall into genomics. When human genome sequencing was completed and compared with yeast chromosomes, it was revealed that a reasonable bulk of yeast DNA sequences were found in human genome. Genome wide investigations can help in understanding the ability of humans and other organisms to resist certain diseases caused by different pathogens such as virus, bacteria and parasites.
Genome sequencing helps in mapping the chromosomes and developing complete genome sequences.
Proteomics reveals important details of gene expression during infections and different environmental and other conditions that can trigger changes in the micro environment of cells. Genomics and proteomics combined together shows promising results with the possibility to generate new vaccines and other therapeutic proteins in prevention and treatment of many incurable infectious diseases such as HIV, HCV etc.

Genomics is the study of the complete genome of an organism. Proteomics is a branch of molecular biology which studies the complete protein set expressed in a cell in order to understand the structure and function of proteins and how proteins affect the cell processes. Genomics cannot explain the actual conditions of the cells due to the post-translational modifications that occurred during protein synthesis. Hence, proteomics is important to understand the actual conditions and the functions of the cells. This is the difference between genomics and proteomics

Gene therapy

Gene therapy is defined as addition of a functional group or group of genes to a cell by gene insertion,

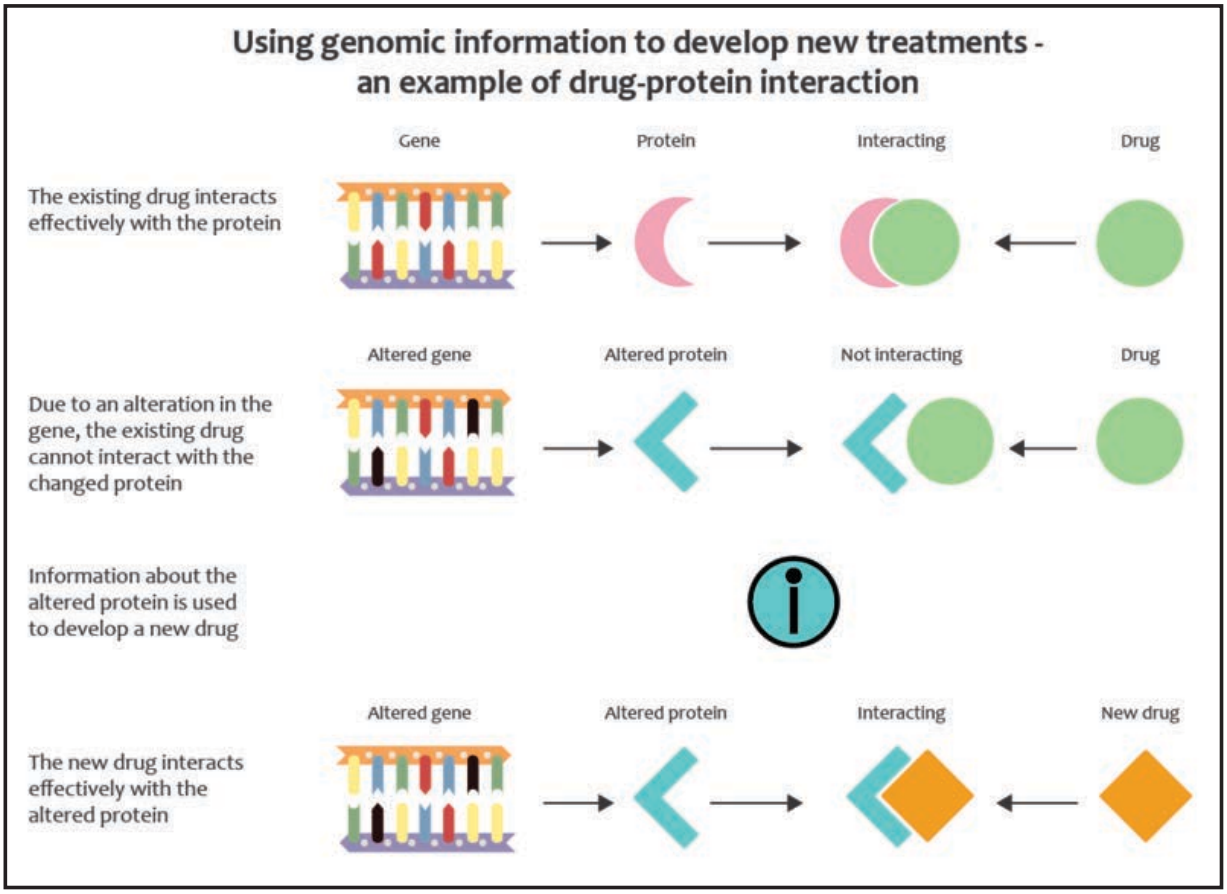


What is hidden inside the Human chromosomes is no longer a mystery.

The human genome project sequenced all three billion base pairs in the human genome. And at the completion of the project it was compared to be an equivalent of placing man on the moon. Today, Gene Editing is far more advanced and would be an equivalent to inhabiting another planet by humans

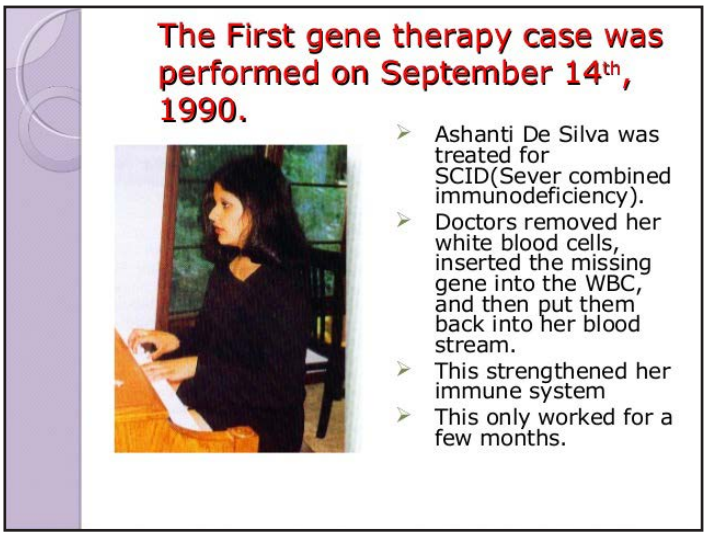
to correct a hereditary disease. In the early stages of gene therapy research applications, the delivery of the new gene was done by using a vector such as a virus without the ability to infect the person, but has the mechanism to insert the incorporated gene. The very first gene therapy treatment on a human was done on a Sri Lankan girl named Ashanthi de Silva.

Gene therapy has changed dramatically in the last 28 years since the first human gene transfer experiment.
All aspects of the procedure has improved and advanced making it possible for people all over the world to access the knowledge related to these technologies. Identifying a specific genetic defect has become less time consuming, specific and simple to apply. Micro arrays can scan hundreds and



thousands of person's genetic information within minutes to few hours. In a similar manner, micro arrays can scan a large number of patients for the same disease within a very short time with precision and accuracy. Precise detection is the first step in gene therapy and this is becoming more and more reliable and accessible to people.

Once the defect is identified, the correct gene can be synthesized or copied from a healthy chromosome. The polymerase chain reaction (PCR) has been the wonder tool for many years for reproducing large numbers of short DNA fragments with the same base sequence.Since the 1983 discovery of a Nobel Prize winning idea by Karry Mullis, different scientists have added many features to PCR, making it possible to be used in disease diagnostics, DNA finger printing, Forensic DNA technology, genome sequencing, recombinant DNA technology and many more gene technology applications. Since the delivery system is the vital part of the whole treatment procedure, the success of gene therapy has largely been driven by improvements in non-viral and viral gene transfer vectors. An array of physical and chemical non-viral methods have been used to transfer DNA and mRNA to mammalian cells, and a substantial number of these have been developed as clinical stage technologies for gene therapy, both *ex vivo* and *in vivo*.With nanotechnology having a say in every other technology applied today, Nano-bio technology is showing the ability to target deliver genes using Nano particles designed for the purpose. Two different methods are used in delivering the correct gene



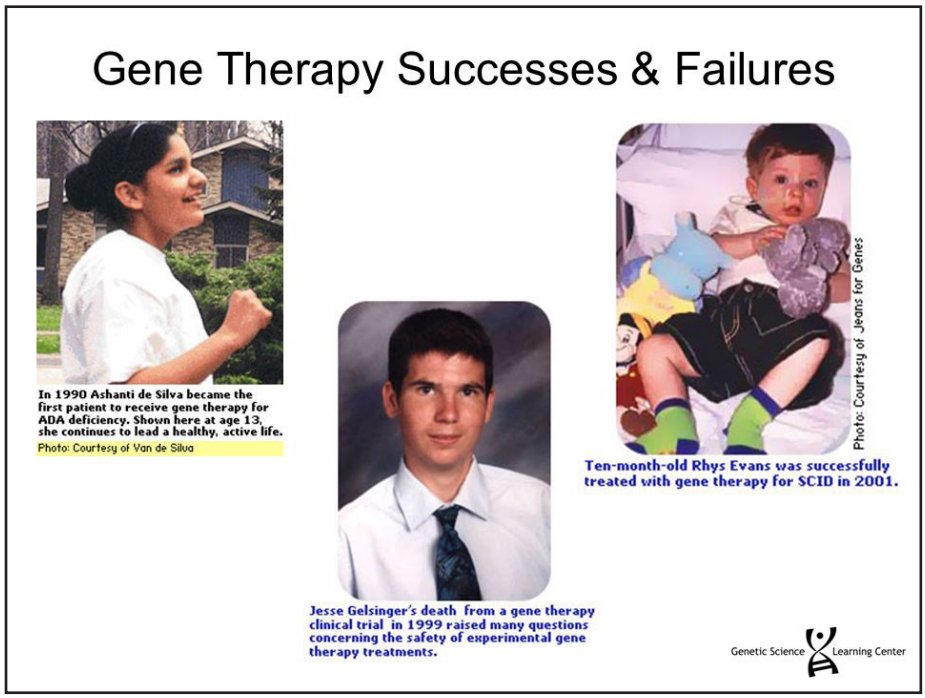
1. Transduction - Transfer via the viral vectors
2. Transfection - Transfer via the non-viral vectors

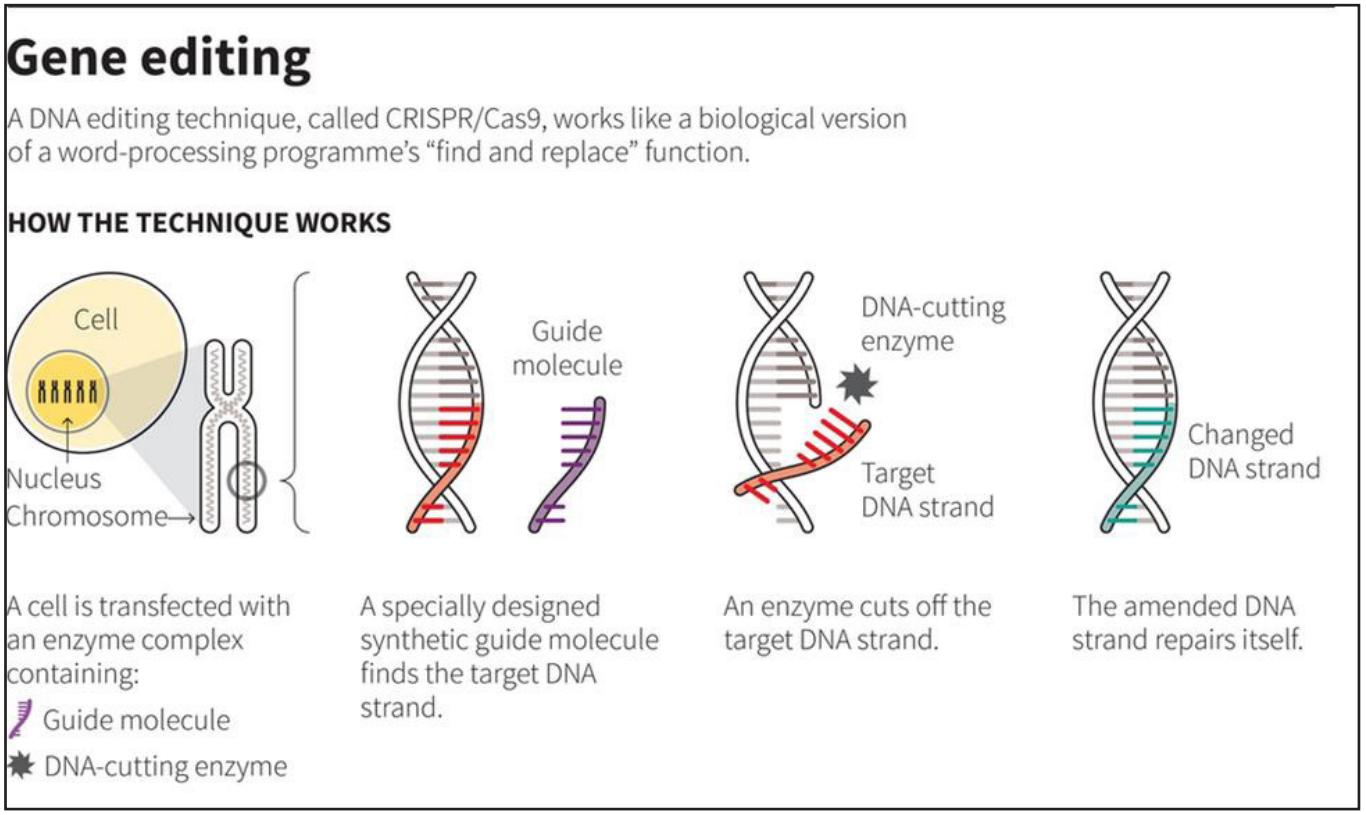
Transduction

Transduction is considered to be the more promising system of gene delivery with various advantages over physical and chemical methods:
Gene transfer is more efficient and specific than physical and chemical methods. Multiple and repeated doses are required in the case of physical and chemical methods, whereas in the case of viral vector, even a single dose is sufficient.
The biggest draw- back is that the vector can harm the patient in certain instances.

Transfection

Non-viral vectors include naked-DNA and liposomes. They are based on plasmid, which is a closed, circular DNA strand. Therapeutic genes can be inserted directly into the plasmid, and then this recombinant plasmid can be introduced into cells in a variety of ways. For example, it can be injected directly into targeted





tissues as naked-DNA.

They are considered safe, since there is no possibility of recombination that would result in a competent virus that could potentially cause disease. The "gene-gun," does not require the presence of complicated and potentially toxic delivery systems. Gold particles bound to the DNA fragments are shot into the cell under high pressure and speed giving the ability to travel through the cell membrane and nuclear membrane. But has less efficient gene transfer rate than transduction.

Rapidly Improving Tools

The technology is often known as CRISPR/Cas9, pronounced "crisper".

In 1987, Japanese scientists studying E. coli first came across some unusual repeating sequences in the bacteria's DNA. "The biological significance of these sequences," they wrote, "is unknown." Over time, other researchers found similar clusters in the DNA of other bacteria (and archaea). They gave these sequences a name: ***Clustered Regularly Interspaced Short Palindromic Repeats — or CRISPR.***

Later it was found that when bacteria are under constant assault from viruses, they produce enzymes to fight off viral infections. Whenever the bacteria's enzymes manage to kill off an invading virus, other little enzymes will come along, scoop up the remains of the virus's genetic code, cut it into little bits, and then store it in those CRISPR spaces.

The bacteria use the genetic information stored in these CRISPR spaces to fend off future attacks. When a new infection occurs, the bacteria produce special attack enzymes, known as Cas9, that carry around those stored bits of viral genetic code like a mug shot. When these Cas9 enzymes come across a virus, they see if the virus's RNA matches what's in the mug shot. If there's a match, the Cas9 enzyme starts chopping up the virus's DNA to neutralize the threat.

Scientists later discovered they could "fool" the Cas9 protein by feeding it artificial RNA a fake mug shot. When they did that, the enzyme would search for anything with that same code, not just viruses, and start chopping. In a landmark 2012 paper, Doudna, Charpentier, and Martin Jinek showed that they could use this CRISPR/Cas9 system to cut up any genome at any place they wanted

These rapidly improving tools

were discussed in the International Summit on Human Gene Editing, which was started to explore the many questions surrounding the use of gene editing tools in humans. The U.S. National Academy of Sciences, the U.S. National Academy of Medicine, the Royal Society, and the Chinese Academy of Sciences hosted a three-day international summit on December 1-3, 2015, inWashington, DC. In this summit it was pointed out that the new gene editing tools are the product of more than 60 years of fundamental research into the molecular nature of DNA molecules.

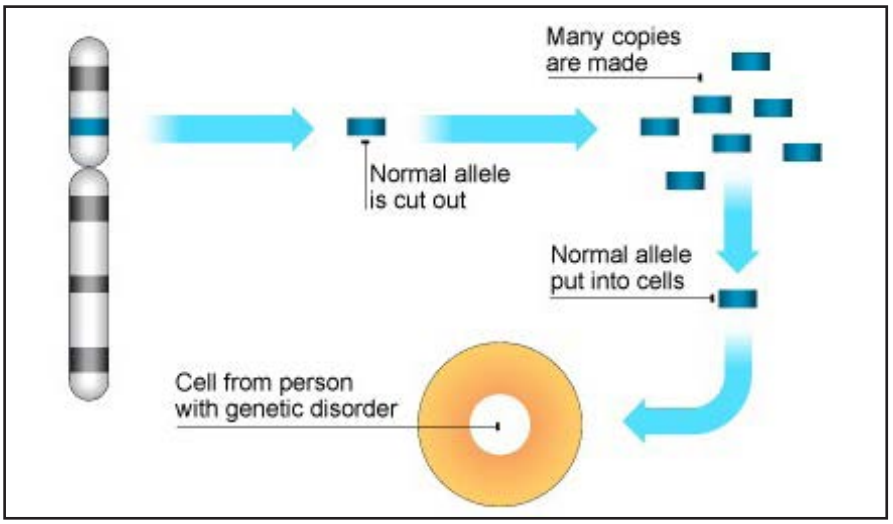
Previous technologies using molecules known as zincfinger nucleases and TALENs had made it possible to alter DNA at targeted locations. While these technologies are currently being used in clinical trials, they are cumbersome and difficult to use.

Therefore new and simple ways of altering targeted genes effectively and precisely were needed, and a new technique using a molecular assemblage known as CRISPR-Cas9, which arose out of research into how bacteria protect themselves from viral infection, is simple, inexpensive, and can target DNA sequences with great specificity.

In 2017 alone, researchers reported in Nature that they had successfully used CRISPR in human embryos to fix a mutation that causes a terrible heart muscle disorder called hypertrophic cardiomyopathy.

"The system is so overwhelmingly efficient and specific that it is changing our entire outlook for future gene editing," - Rajewsky.

Still , CRISPR-Cas9 needs to be perfected, as it can alter DNA at locations other than the target leading to inactivation of essential genes, activation of cancer-causing genes or chromosomal rearrangements. Effectiveness of the system may be efficient in certain type of cells but not all, resulting in a mosaic of altered and unaltered cells. Some scientists are cautious that it can generate immune responses if introduced into the body. There are many drugs that are in use which cause off-target effects, but are still effective for the targeted treatment. Similarly the CRISPR-Cas9 system is still undergoing development to reach the level of safety where it could be used in clinical applications and become a safe and approved treatment for currently incurable diseases.



Basic concept in Gene therapy includes identifying the defective gene, finding the correct sequence for it, making large number of copies of the correct gene and delivering the correct gene to the target.
Delivering has been the major concern in application of the technique right through the years.As different vectors sometimes had their own negative impacts on the patients after the treatment.

Ms Vajirapani De Silva,
Senior Scientist
Gene Tech
vajirapani@genetechsrilanka.com
0112696992, 0777974370