Genome Editing Techniques: A survey

Srushti Honnenahalli

E-mail: srushti1013@gmail.com

Abstract:

The spring of genome engineering technology has opened new avenues into the arena of molecular medicine and has become a powerful genetic tool for studying gene functions and for modifying genomes by correcting the defective genes or introducing genes. The expansion of genome editing technologies has opened up the opportunity to directly target and change genomic sequences in most eukaryotic cells. Genome editing has increased our ability to understand the relationship of genetics with pathogens by creating more accurate and effective cellular and animal models of pathological processes and shows mass potential in a variety of fields like, basic research, applied biotechnology, and biomedical research. The initial molecular genome-editing techniques that had emerged were mega nuclease, transcription activator-like effector nucleases (TALENs), and zinc-finger nucleases (ZFNs). Following these advancements, the latest discovery of molecular technique is CRISPR/Cas9 technology, which is a genetic manipulation tool derived from the immune system of certain bacteria to protect themselves against viruses and plasmids (pathogens). This mentioned technique is rapidly progressing into clinical trials because of its better efficiency and feasibility. Genome editing is a powerful technique for biomedical research, and it assures altering inherited diseases. This review will outline the various genome-editing technologies that are currently available, some of the challenges in the implementation and limitation of these technologies, and ethical issues surrounding this field of biotechnology.

Keywords: Genetic techniques; Mega nuclease; transcription activator-like effector nucleases (TALEN); Zinc finger nuclease (ZFN); CRISPR/Cas9; genome editing

1. Introduction:

One major challenge in biotechnology has been developing efficient and reliable ways to make targeted changes within the genome of cells. The development of genome editing has majorly changed research on the human genome, which enables analysts to understand the contribution of a single-gene product to disease in an organism. The initial Genomic editing initially produced in yeast and mice in the 1970s and 1980s[1]. Traditional methods of using chemical reagents for mutagenesis may require extensive screening to restore the desired mutation[2]. The gene can be inactivated by the process of homologous recombination to determine the gene function. That strategy can be implemented into cultured cells by a process called transfection, which forces the introduction of small molecules such as DNA, RNA, or antibodies in eukaryotic cells[3]. Current approaches in genome editing support manipulation of any gene at its center in a wide variety of species and tissues. Genome editing depends on engineered nucleases to cut specific genomic target sequences and changes the region of the genome by introducing double-strand breaks (DSBs), which is corrected by cellular tools. Genome-editing techniques are useful in small gene mutations, sectional removal of DNA, and sectional insertion in DNA. Programmable nucleases consist of a nuclease section that cuts the DNA, and A DNA-targeting segment intended to lead the nuclease to a specific sequence of DNA.

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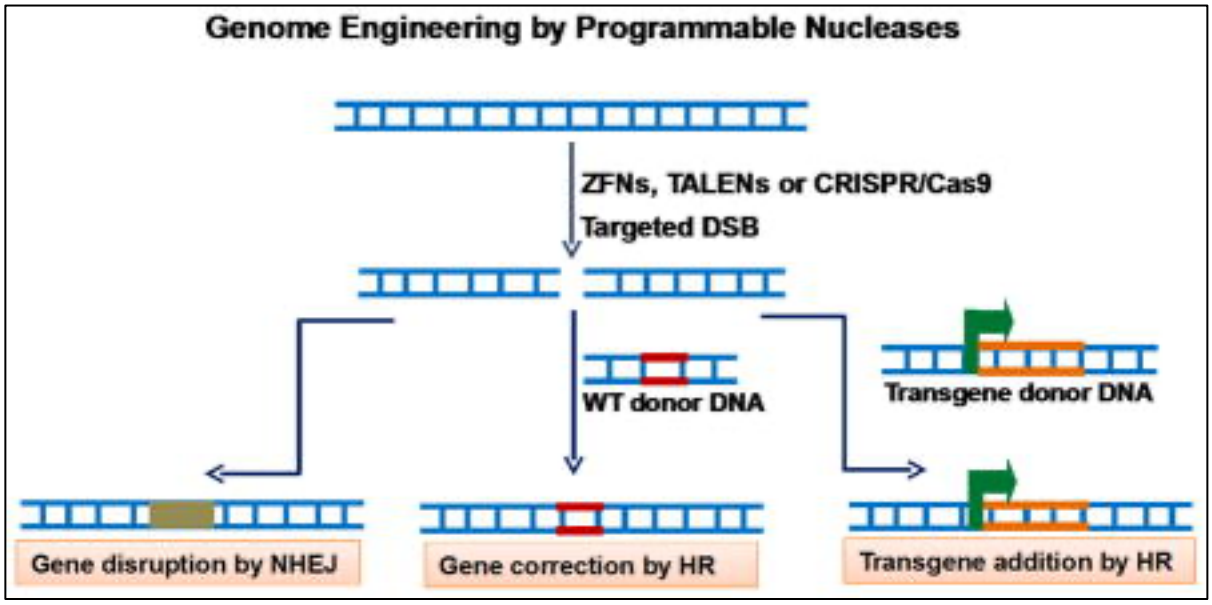
[1] Rothstein RJ. One-step gene disruption in yeast. Methods Enzymol. 1983; 101: 202–11.

[2] J.M. Chalker, B.G. Davis. Chemical mutagenesis: selective post-expression interconversion of protein amino acid residues. Curr Opin Chem Biol, 14(6) (2010), pp. 781-789.

[3] Im W, Moon J, Kim M. Applications of CRISPR/Cas9 for gene editing in hereditary movement disorders. J Mov Disord. 2016; 9:136–143. doi: 10.14802/jmd.16029.

After the 'cutting' process, the DNA in that particular place would naturally repair the cut region. The repairing process will then be manipulated in that location in the genome to make edits to the DNA[4]. Soon by using this biotechnology concept, we will be able to improve the concept of personalized medicine. Thus, making the immune systems stronger to protect itself from harmful pathogens. In this survey, we will go further in detail on the different modern genome-editing tools, comparisons and contrasts, and will identify bioethical concerns regarding genome-editing technologies.



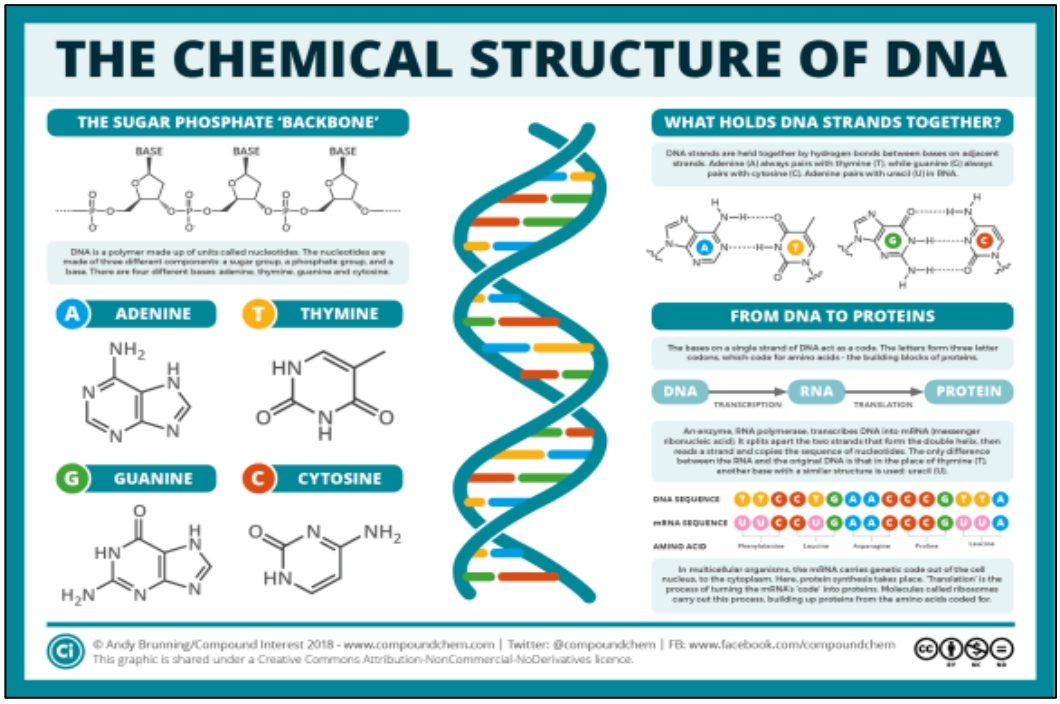
(Figure 1a[14])

2. ***Brief Concept and terminologies***:

2.1.*Deoxyribonucleic Acid (DNA)*:

[4] National Academies of Sciences, Engineering, and Medicine; National Academy of Medicine; National Academy of Sciences; Committee on Human Gene Editing: Scientific, Medical, and Ethical Considerations. Human Genome Editing: Science, Ethics, and Governance. Washington (DC): National Academies Press (US); 2017 Feb 14. A, The Basic Science of Genome Editing. Available from: https://www.ncbi.nlm.nih.gov/books/NBK447276/

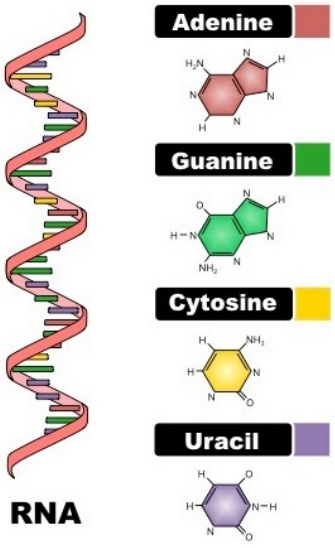
DNA is a double helix of two antiparallel strands held together by hydrogen bonds between complementary purine and pyramidine bases. The information in DNA is stored as a code made up of four chemical bases: adenine (A), guanine (G), cytosine (C), and thymine (T). An important property of DNA is that it can replicate itself. Each strand of DNA in the double helix can serve as a pattern for duplicating the sequence of bases. This is critical when cells divide because each new cell needs to have an exact copy of the DNA present in the old cell[5]. The chemical structure of the DNA is shown in Figure 2.1a.



(The chemical structure of the DNA, 2015, p. Figure 2.1a)

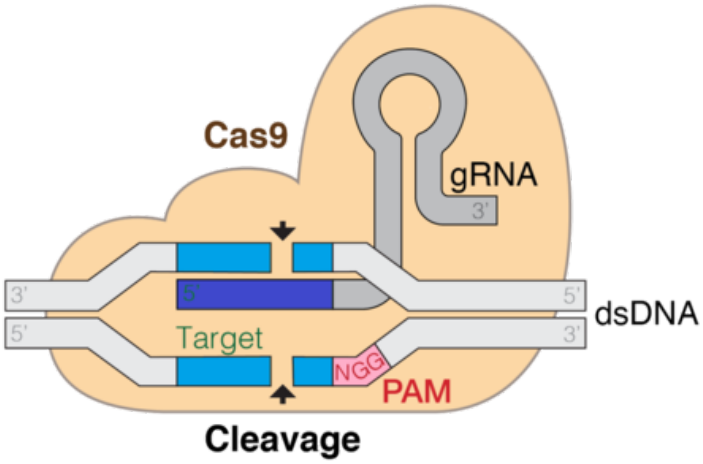
[5] Lodish H, Berk A, Zipursky SL, et al. Molecular Cell Biology. 4th edition. New York: W. H. Freeman; 2000. Section 4.1, Structure of Nucleic Acids. Available from: https://www.ncbi.nlm.nih.gov/books/NBK21514/

2.2. *Ribonucleic acid (RNA)*:



Unlike DNA, RNA is a single-strand and is made of alternating sugar (ribose) and phosphate groups. Attached to each sugar is one of four bases: adenine (A), uracil (U), cytosine (C), or guanine (G). There are several kinds of RNA in the cell: messenger RNA (mRNA), ribosomal RNA (rRNA), and transfer RNA (tRNA). Sequence variations in RNA maintain base-pairing patterns that give rise to double-stranded regions (secondary structure) in the molecule. These secondary structures are formed by folding the single-stranded molecule back upon itself[5]. (Figure 2.2[6])

2.3. Programmable Guide RNA (gRNA):



A guide RNA directs the function of a CRISPR protein effector to a target gene of choice, providing a versatile programmable platform for engineering diverse modes of synthetic regulation[7]. (Figure 2.3a[8])

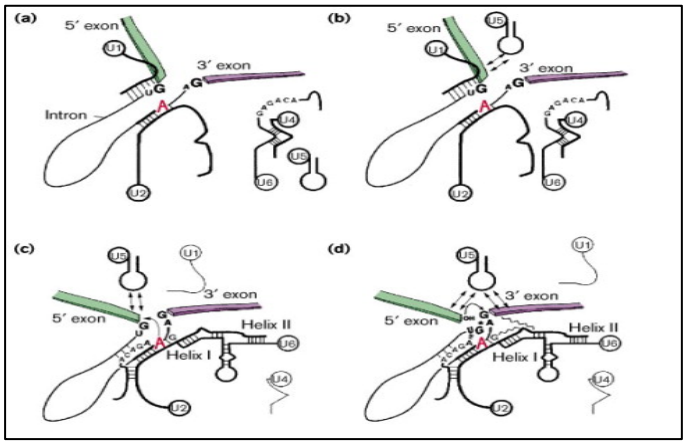
[6] RNA Structure, 2018

[7] Mikhail H. Hanewich-Hollatz, Zhewei Chen, Jining Huang, Lisa M. Hochrein, Niles A. Pierce. Conditional Guide RNAs: Programmable Conditional Regulation of CRISPR/Cas Function in Bacteria via Dynamic RNA Nanotechnology bioRxiv 525857; doi: https://doi.org/10.1101/525857

[8] (Gerardduenas, 2016)

2.4. *Splicing Method*:

Splicing occurs in spliceosomes, 'which are large particles that are built-up stepwise on the mRNA precursor from smaller RNA–protein sub-assemblies called snRNPs (small nuclear ribonucleoprotein particles)'[9]. In the spliceosome, each snRNPs contains general set of snRNP proteins. Errors during mRNA splicing will have catastrophic biological effects.

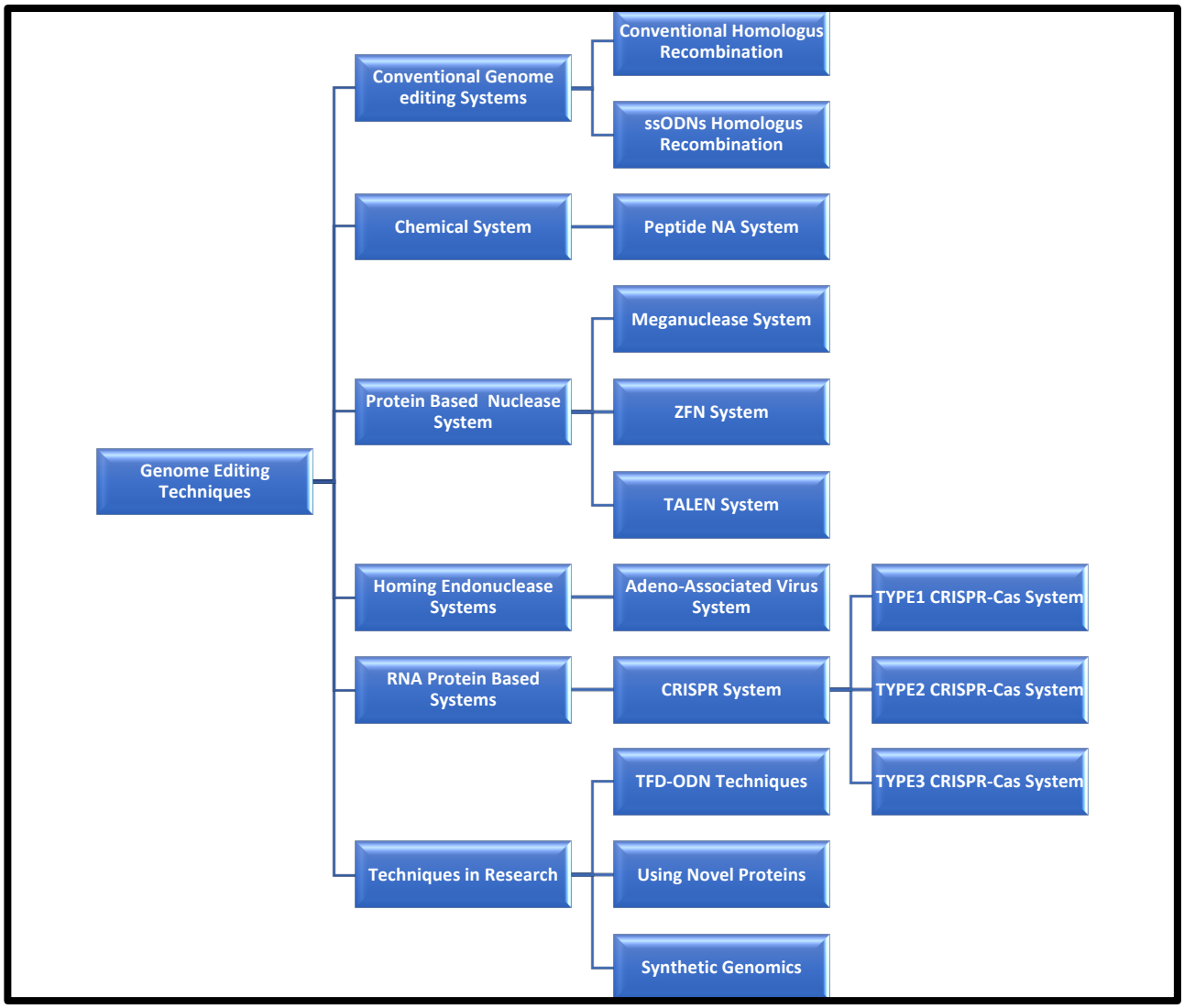


(Figure on left: (Newman, n.d.))

3. Genome Editing Techniques:

The expansion of this biotechnological field has not only improved and updated the older genome editing techniques but has advanced in creating efficient modern tools which are used in clinical trials. The flowchart has summarized the different genome editing techniques to make it simpler to understand (Khan 2019).

[9] Newman, A. (n.d.). RNA Splicing. Retrieved May 2, 2020, from https://www.cell.com/current-biology/comments/S0960-9822(98)00005-0



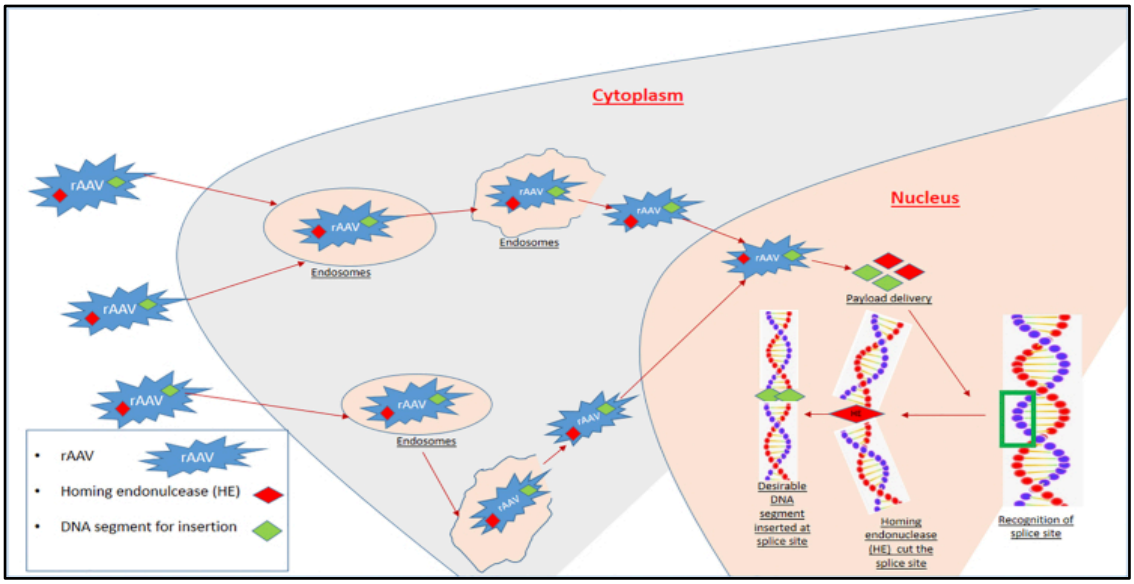
The modern genome editing techniques mentioned in the chart will be discussed in detail below:

3.1. *Homing Endonuclease Systems*:

Meganucleases or homing endonucleases (HEs) are sequence-specific endonucleases with large (>14 bp) fractured sites that are used to produce efficient homologous gene targets in cultured cells and plants. Human cell and tissues that are affected by a variety of diseases. Recently recombinant adeno-associated viral (rAAV) vectors has been introduced to treat human disorders. As shown in Figure 3.1a (Khan, 2019), rAAV are non-pathologic and effective means of viral and non-viral gene

transportation[10]. However, the clinical applications of rAAV are limited due to the presence of immune responses in the host organism and the existence of physiological barriers[10]. The steps below show rAAV entry and movement shown the Figure 3.1a (Khan, 2019):

a. Entry of rAAV into cell
b. uptake by exosome and transport within the cytoplasm
c. rAAV is released into the nucleus
d. rAAV delivery of homing endonuclease (HE) and desirable DNA segment
e. Homing endonuclease (HE) cuts the non-desirable segment of DNA code
f. Replacement of the DNA with the delivered desirable DNA code



3.2. *Protein Based Nuclease System*:

Protein Based Nuclease Systems incorporate nuclease proteins for DNA sequence editing. The common techniques are:

[10] Khan, Sikandar Hayat. "Genome-Editing Technologies: Concept, Pros, and Cons of Various Genome-Editing Techniques and Bioethical Concerns for Clinical Application." *Molecular therapy. Nucleic acids* vol. 16 (2019): 326-334. doi:10.1016/j.omtn.2019.02.027

3.2.1. *Meganucleases Technique*:

Meganuclease are naturally occurring endonucleases distinguished by large recognition sites, which are unique in most genomes. Also, they have specific splice fracturing due to their naturally occurring properties. They are important tools for genome engineering because they provide an efficient way to generate DNA double-strand breaks at specific loci of interest[10]. This technique has two primary steps: the recognition of a fracture site, and the endonuclease splicing the region[11]. This technique hasn't been updated because the focus is on creating newer and more efficient tools.

3.2.2. *Zinc-Finger Nuclease (ZFNs)*:

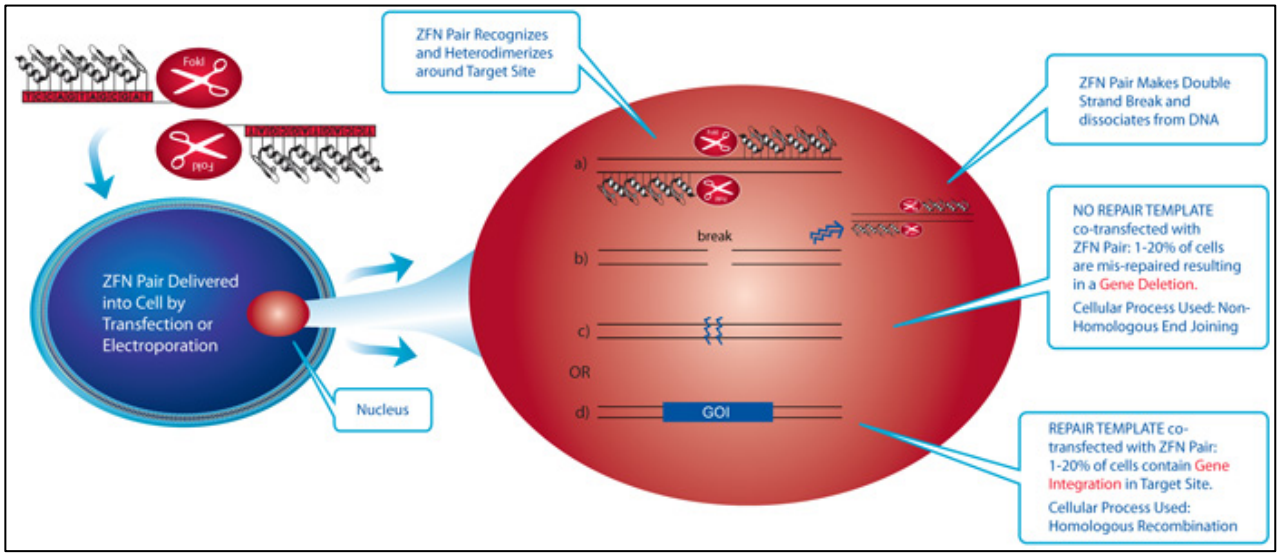
ZFNs are a class of engineered DNA-binding proteins, which promotes targeted editing of the genome by creating double-strand breaks in DNA at specified sections. This technique is being used in clinical applications for certain diseases due to its specificity, efficiency, and simplicity[12]. ZFNs mechanism contains FokI endonucleases and protein-binding domains. These are introduced into the cell. Then the FokI and protein-binding domains are released to enter the nucleus. These protein-binding domains attach themselves with the DNA fragment that is to be removed. Next, the FokI creates double stranded DNA breakage, which causes the identified DNA segment to cut. Finally, the desired DNA segment is inserted and combined

[11] McMurrough T.A., Brown C.M., Zhang K., Hausner G., Junop M.S., Gloor G.B., Edgell D.R. Active site residue identity regulates cleavage preference of LAGLIDADG homing endonucleases. Nucleic Acids Res. 2018;46:11990–12007.

[12] Ochiai H., Yamamoto T. Construction and Evaluation of Zinc Finger Nucleases. Methods Mol. Biol. 2017;1630:1–24.

into the DNA sequence. Thus, creating a more desirable DNA segment[12].

Attached below is an image of ZFN technique (Figure 3.2.2a[13]).



3.3. *Transcription Activator-like Effector Nucleases (TALEN) Technique*:

TALENs are similar in principle with ZFNs, however, TALENs are more site specific with fewer off-targets effects. TALENs mechanism starts with FokI endonucleases and TALE domains that will be introduced into the cell. Then to enter the nucleus, FokI and TALE domains are released. Next, TALE domains attach themselves with the recognized non-desirable DNA segments. FokI will proceed to split with the non-desirable DNA segments, and the desirable segment of DNA is incorporated into the DNA[14].

3.4. *RNA Protein Based System*:

This system contains different types of CRISPR-Cas systems. CRISPR stands for Clustered Regularly Interspaced Short Palindromic Repeats and Cas stands for

[13] Zinc-Finger Nuclease (ZFNs) technology, Google Image.

[14] Chandrasegaran S., Carroll D. Origins of Programmable Nucleases for Genome Engineering. J. Mol. Biol. 2016;428(5 Pt B):963–989.

CRISPR-associated. Editing requires two elements: a Cas nuclease and a programmable guide RNA. There are three stages of CRISPR/Cas9: Adaptation, expression, and interference. To destroy an antigen, CRISPR RNA guides the Cas9 protein to the threat location and creates a double-stranded DNA. Then, it cuts the guided sites, which causes a fracture or a break, and therefore destroying the antigen and storing its memory as a Spacer within the CRISPR[15].

CRISPR/Cas9 technology has attained widespread popularity due to its simplicity and specificity. After many experimentations CRISPR/Cas technologies are now classified into three major types due to its CRISPR RNA processing. The three types are as follows:

3.4.1. *Type 1 CRISPR/Cas system*:

For pre-processing of crRna (CRISPR RNA), Cas5 or Cas6 versions are used. Also, for further fracturing, the function requires Cas3, Cascade, and crRNA for interference[10].

3.4.2. *Type 2 CRISPR/Cas system*:

This Cas9 stem requires: RNase III, trans-activating RNA (tracrRNA), and a soon-to-be-identified protein to be involved in trimming at the 5′ end.

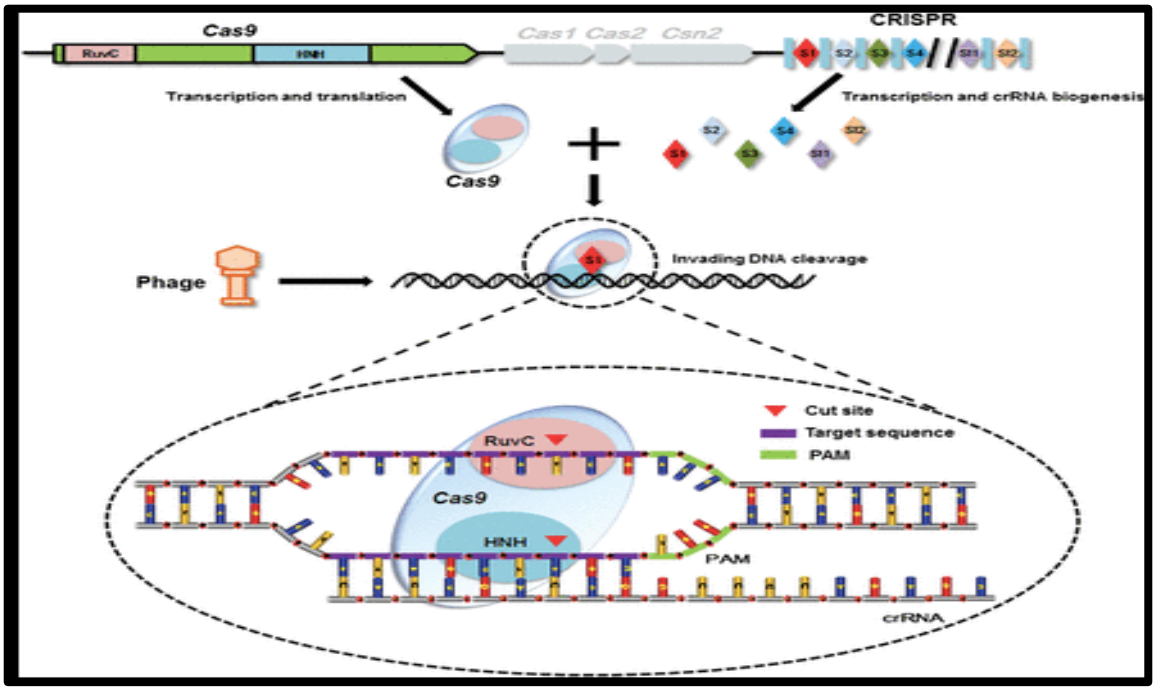
3.4.3. *Type 3 CRISPR/Cas system*:

This type also uses Cas6 for processing crRNA 3′ end trimming. The difference between Type 1 and Type 3 is that it uses specific complex called type III Csm/Cmr complex to identify and target the RNA[16].

[15] Zhang F., Wen Y., Guo X. CRISPR/Cas9 for genome editing: progress, implications and challenges. Hum. Mol. Genet. 2014;23(R1):R40–R46.

[16] Rath D., Amlinger L., Rath A., Lundgren M. The CRISPR-Cas immune system: biology, mechanisms and applications. Biochimie. 2015;117:119–128

Scientists are keen to find better ways to ensure that the CRISPR-Cas9 binds and cuts more efficiently. Currently CRISPR-Cas9 is used on plants and animals. With further research, soon CRISPR-Cas9 can be used on humans ethically to defend the body against harmful pathogens. (Figure 3.4a[16]) Though research work in this domain stands preliminary, over time it is expected to overtake the idea of genome editing.



(Figure 3.4a[16])

4. ***Comparative Analysis***[10]:

Table 1: Biotechnology Differences among Prototype Genome-Editing Techniques

TABLE 1:	**Parameter**	**ZFN**	**TALEN**	**CRISPR/CAS**

1.	Design simplicity	Moderate	Slightly complex	Simpler
2.	Engineering feasibility	low	high	highest
3.	multiplex genome editing	Few models	Few models	high yield multiplexing available
4.	large-scale library preparation	Not much progress	Not much progress	progress demonstrated
5.	specificity	low	high	highest
6.	Efficiency	Normal	Normal	High
7.	Cost	Low	High	Low

Table 2: Side Effect Profiles for Genome-Editing Methods

TABLE 2[10]**:**	Parameter	ZFN	TALEN	CRISPR/CAS
1.	Off-target effect incidence	-	-	-
	a. homologous recombination rate frequency	+	+	+
	b. Non-homologous end joining	+	+	++

	(NHEJ) mutation rates			
	c. Immune reaction susceptability	Less	Less	More
	d. RGEN Induced off-target mutatagenesis	-	-	++
2.	Cytotoxicity chances	++	+	+

Table 3[10]: Clinical and Research Applications across Important Genome-Editing Techniques

Table 3:	Parameters	ZFN	TALEN	CRISPR/CAS
1	Diagnostic utility	+	++	+++
2	Clinical trial use	++	+	+++
3	Epigenetic maker utility	++	+++	++++
4	Making gene-knockout methods models for research	No	No	Yes (CRISPRi)
5	capacity for modification of	No	No	Probable

	mitochondrial DNA			
6	Genetic editing in human babies	No	No	Yes
7	RNA editing	No	No	Yes

5. Bioethical Issues:

Genome-editing techniques are powerful enough to bring about biotechnological revolution in the field of agriculture advancement and human pathology. These tools could help human strengthening and increasing the pace of human evolution to dangerous diseases and injuries. However, this can be a positive path if research and experimentation is done ethically. Unethical approach using these biotechnologies can lead to misuse of these tools causing manipulation of germline genetics. Many experts state that these editing tools can become a future weapon of war. This is catastrophic because it can take the concept of "survival of the fittest" to another level of dread. Apart from illegal germline mutations, other concerns are about clinical debates on informed consent, religious debates, negative impacts of creating "superhumans," and designed babies[10]. Designer babies are currently being debated, especially after the He Jiankui affair. In Nov. 25, 2018, He Jiankui had used CRISPR to edit embryos, two of which had in October 2019, became living babies: Nana and Lulu. They were born prematurely by emergency C-section. He had injected CRISPR to cause 32-base-pair deletion in a gene called CCR5, which is found in chromosome 3. This prevents the babies from having

AIDS. However, the cells had modified and creates the production of non-functional protein, that had never been seen in humans before. Although, He says that there haven't been any other changes in the gene[17]. While the quest for healthy babies is understandable, it is not ethical as there is much to learn about these tools and this could become the cause of harming babies' lives. We need regulated translation to protect our species and the genetically modified crops. These GMOs could cause more damage than benefit the population, so, it must be handled in the right way.

6. Conclusion:

Gene-editing technology has promoted the development of cell imaging, gene expression regulation, epigenetic modification, and gene diagnosis[16]. Thus, bringing genome editing technology closer to the clinic. This review has discussed various genome-editing technologies, basic concepts on mechanisms, comparisons between the major genome technologies, and has discussed the bioethical issues. Although CRISPR/CAS has exceeded the initial expectations of modern genome editing systems, it is being constantly updated and improvised. Also, by the rate these technologies are advancing there will soon be newer and more efficient editing technologies that can replace CRISPR/CAS tool someday. At the same time the issues related to bioethics need to be considered and acted upon to preserve the basic human rights and to ensure it doesn't become a biological weapon or a world crisis.

[17] Greely HT. CRISPR'd babies: human germline genome editing in the 'He Jiankui affair'. *J Law Biosci*. 2019;6(1):111-183. Published 2019 Aug 13. doi:10.1093/jlb/lsz010