CODEN (USA): IAJPBB ISSN: 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES

http://doi.org/10.5281/zenodo.344030

Available online at: http://www.iajps.com **Review Article**

A REVIEW ON GENOME EDITING

Akshay Patil, Latesh Patil, H. P. Suryawanshi, S. P. Pawar
Dept. of Pharmacology, P.S.G.V.P. M.'s College of Pharmacy, Shahada, (M S).

Received: 28 January 2017 **Accepted:** 10 February 2017 **Published:** 28 February 2017

Abstract:
The Present Review report contains the information about Genome Editing. In this report genome editing is illustrated at the basic level for better understanding. It contains History of Genome Editing from 1800 to Current day i.e. from concept of DNA till current time. Genome editing is an technique to make every human smarter not our choice, by customizing next generation the way we want it to be like to be look and also the intellectual capacity. In this Concept, the viral Immunity is used to treat untreated diseases by using DNA modification technology. Most common and easy way of Genome editing that is CASPER/Cas9 is highlighted in short and other methods like Meganeucleus, Transcription activator-like effectors nucleases, Zinkfiger nucleus all are described in short. The Report also contains flow charts of Targeted mutagenesis using embryonic stem (ES) cells for better understanding. The review report also contains Genome Editing in not only in Humans but in Plants also. Which will give us better Crop yield with greater quality of food and containing more amount of active ingredients. For plants there are points taken which are, The importance of mutants in gene discovery, Agrobacterium-mediated gene-tagging mutagenesis etc. and also a flow chart of Plant Genome editing using CASPER/Cas9 technique. Now at the last but not the lease, Advantages and Disadvantages of Genome Editing to know that what this technique will give us in future and what it will take away from us.

Key Words:*Meganucleus,nucleases, Agrobacterium, Genome, CRISPER/Cas9.*

Corresponding Author:
Akshay Patil,
Dept. of Pharmacology,
P.S.G.V.P. M.'s College of Pharmacy,
Shahada, (M S).
Email ID: meaksh49@gmail.com
Contact No.: 07588733330

QR code

Please cite this article in press as Akshay Patil et al, ***A Review on Genome Editing****, Indo Am. J. P. Sci, 2017; 4(02).*

INTRODUCTION:

"Instead of creation of offspring by chance we can create offspring by choice."

1. It seems as though genome editing is everywhere. In a relatively short time, particularly since the emergence of the CRISPR-Cas9 system in 2012, techniques for making precisely targeted alterations to DNA sequences in living cells have not only preoccupied life science journals, but have also featured in mainstream news. They have been implicated in stories of revolutionary medical advance and genetically altered food, and in the business pages, where the battle over the intellectual property rights to the underlying technology, and the prospects of companies developing genome editing treatments and products, have been matters of continual intrigue and speculation.

2. While the scientific merits are overt, the practical and ethical significance of these recent developments is far harder to discern. While the use of genome editing techniques has spread across biological research, including microorganisms, plants, animals and human cells, the extent to which the potential applications can be understood in relation to prevailing norms and managed through existing governance measures has not been extensively examined. As a rapidly established (though continually developing) research technique, one that is at the foundation of diverse emerging biotechnologies, there is concern that genome editing science and innovation are moving ahead of public understanding and policy.

Concept:

People have long sought and used scientific knowledge to improve the conditions of human life. Contemporary molecular biology affords a particularly powerful set of tools that form the basis of a range of technologies in fields as diverse as medicine, agriculture, industrial production, and environmental management. What we will refer to as 'genome editing' is the practice of making targeted interventions at the molecular level of DNA or RNA function, deliberately to alter the structural or functional characteristics of biological entities. These entities include complex living organisms, such as humans and animals, tissues and cells in culture, and plants, bacteria and viruses. Characteristics of many kinds, from the colour or number of blooms in flowering plants, to some disease traits in animals and plants, can be altered, though the extent to which, and ease with which, such alterations can be made is highly variable.

History:

Late. 1860 – The Discovery of DNA by Friedrich Miescher[1]

Late 1880s- Phoebus Levene did extensive research about the DNA molecules[1]

1920s- Erwin Chargaff discovered the primary chemical components of DNA and the way that they are Attached to one another[1].

1953- James Watson and Francis Crick found the three-dimensional double helix structure of DNA[2]

1970s- Frederick Sanger, as well as the contributions from many other scientists and organizations, were able to independently invent a method of genome sequencing.[2]This was not only able to read DNA, but increase the affectivity and decrease the cost and time of genome sequencing.[1]

Around 1975- The Sanger Method, which is also known as the Chain Termination Method, evolved into the method of "shotgun" sequencing (described in "Present Technology"). Shotgun sequencing caused genome sequencing to become much quicker and to be the most widely used method[1]

1983- Kary Mullis invented the Polymerase Chain Reaction (PCR). The PCR is able to make many copies of DNA segments in a simple and inexpensive way, such as diagnosing diseases, identifying bacteria and viruses, and recognizing criminals for crime scenes[1] .

1984- The U.S. Department of Energy (DOE), National Institutes of Health (NIH), and international groups held conferences to discus the human genome[2]

1988- The idea of mapping the human genome was presented in order to find genetic maps, physical maps, and the complete nucleotide sequence map of the human chromosomes[2]

2003- Scientist were able to accurately map the human genome[1]

September,2012- Genome editing began with the discovery of epigenetic editing.[3] Epigenetics is able to shape the structure of the genome by tightly wrapping inactive genes to making them unreadable, and relaxing active genes to make them readable. While DNA is the same all of your life, epigenetics are flexible based on signals from outside sources[4]

April, 2013- The CRISPR/Cas system was used on zebrafish[3]

June, 2013- The CRISPR method is used as a user-friendly transcriptional repressor[3]

November, 2013- Epigenetic editing targets DNA demethylation, the process that is able to remove a methyl group from DNA nucleotides, which induces gene expression[3]

February 5, 2014- Chinese researchers conducted experiments on monkeys using the CRISPR/Cas9 method of genome editing.[3]

April, 2014- The CRISPR/Cas9 method was able cure its first human related genes found in mice. This was able to happen by correcting a mutation to create a healthy phenotype.

May, 2014- Genome editing and induced pluripotent stem cells (iPSC) develop a "heart-on-chip" technology to reveals specific mutations of a heart abnormality. A synthetic heart is then created based on that information. Researchers use whole genome editing in human pluripotent stem cell clones to see how much collateral damage the new CRISPR/Cas9 and TALENs nucleus tools present. They found a very low amount of off-target mutations[3]

July, 2014- The CRISPR/Cas9 technology was used in haematopoietic cells and mice. A novel drug inducible lentiviral system was developed to deliver platform cells needed in the methods to cells allowing an easy and rapid way of genome engineering

August, 2014- After combing the CRISPR/Cas9 and ChAP-MS, a new tool was made to see every protein of a specific genomic region.[3]

August, 2014- The short guide RNA (sgRNA) directs the Cas9 to a specific target. The sgRNA was modified to make it reach a wider variety of locations in the genome.[3]

August, 2014- Scientists applied ChIP-Seq to prove that Cas9 can sometimes cause off-target effect.[3]

August, 2014- Patient's specifically induced pluripotent stem cells (iPSCs) are added to the CRISPR/Cas9 method. This allowed the system to meet more specific requirements when editing DNA.[3]

Current Day Genome Sequencing- Genome sequencing technology vary between many manufacturers. A few of the more common sellers of genome sequencers are Applies Biosystems, Illuminia, Roche, Qiagen, Beckman Coulter, and Life Technologies.(5]

What is Genome Editing? [7,8]

A genetic engineering approach in which DNA is inserted, removed or replaced at a precise location within the genome.

- Engineered nucleases.
- Recombination-based approaches
- Creation of isogenic cell lines -> only differ by the change we've introduced.
- Make (nearly) any modification we desire!
- Scientists announced a landmark plan to recreate entire human cells from scratch within the next ten years.
- The enormously complex project involves synthesizing all six billion 'letters' of the entire human DNA code, otherwise known as the genome.
- It could have far reaching implications for the study of diseases such as cancer and even growing replacement organs,sayresearchers.

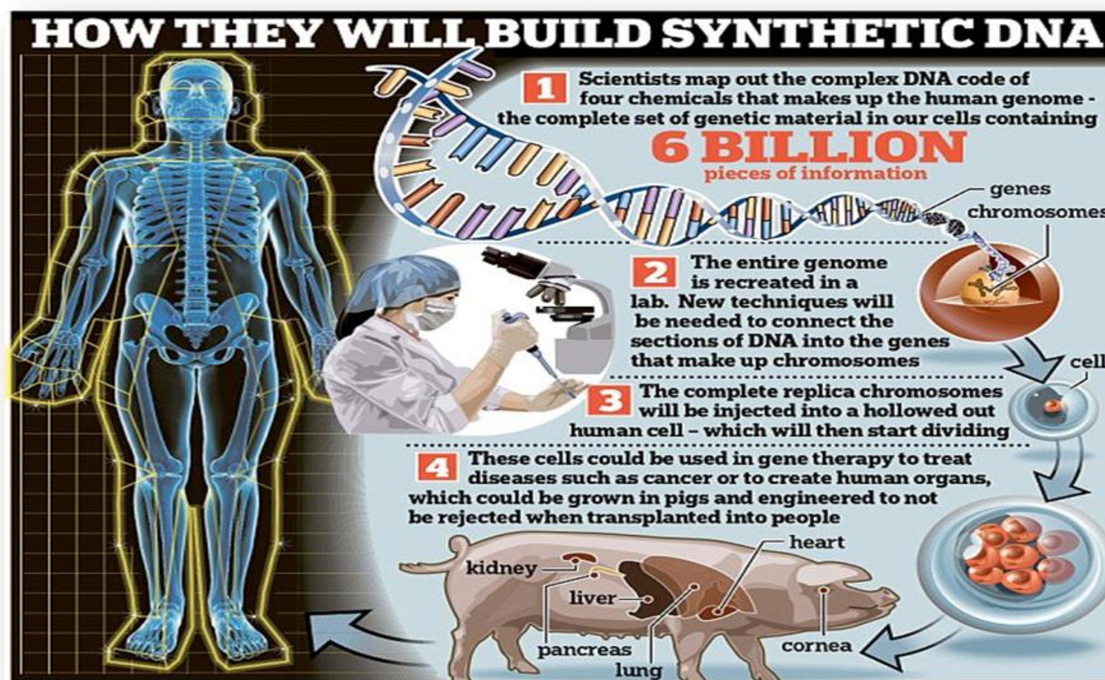


Fig. 1

- Scientists are hoping that the completed DNA, once its made, will be implanted into a living cell and - it is hoped - start to divide.
- At this point scientists will have created, for the first time, a whole human cell of their own design.
- Named the Human Genome Project-write (HGP-write) it could enable researchers to make synthetic human genes and chromosomes for study.
- This could include chromosome 21 – an extra copy of which is responsible for Down's syndrome.
- But implications could extend far beyond, to growing organs for transplant patients, engineering immunity to lethal viruses, such as Ebola or Zika, and even developing cancer-resistant cells in the lab.

What is CRISPER/Cas9?[9,10]

Recently, an efficient, RNA-guided, site-specific DNA cleavage tool, CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats), and the CRISPR-associated (Cas)9 system has been developed from the *Streptococcus pyogenes* type II CRISPR adaptive immune system and has attracted much attention for its potential to transform genome engineering and regulation.

In bacteria and archaea, CRISPR loci usually consist of three components: a cluster of cas genes and two non-coding RNA elements, trans-activating CRISPR RNA (trascrRNA) and a characteristic array consisting of repetitive sequences flanking unique spacer sequences. Each spacer is derived from invading phage or plasmid DNA. Transcription of the array yields individual CRISPR RNAs (crRNAs, consisting of spacer-repeat fragments), which localizes the crRNA: tracrRNA: Cas9 complex to target DNA where the effector Cas9 nuclease cuts both strands of DNA (double-strand breaks, DSBs) that matches the crRNA, and consequently, leads to the inactivation of invading DNA. In mammalian and other cells, CRISPR-Cas induced DSBs can be repaired through two endogenous mechanisms: the non-homologous end joining (NHEJ) method is generally used for the creation of a frameshift deleterious mutation, while the homology directed repair (HDR) is preferred for the introduction of a specific point mutation or addition of genes of interest. This precision targeting feature of the CRISPR-Cas9 system is of great interest for the study of biological processes.

Genome Editing: Tools for CRISPR/Cas9 Application[11,12]:

Genome editing is enabled by the development of tools to make precise, targeted changes to the genome of living cells. Recent approaches to targeted genome modification – zinc-finger nucleases (ZFNs) and transcription-activator like effector nucleases (TALENs) – enable researchers to generate mutations by introducing double-stranded breaks to activate repair pathways. These approaches are costly and time consuming to engineer, limiting their widespread use, particularly for large scale, high-throughput studies. These genome editing techniques were applied concurrently with other approaches to manipulate gene function, including homologous recombination and RNA interference. RNAi, in particular, became a laboratory staple enabling inexpensive and high-throughput interrogation of gene function. However, the utility of RNAi is hampered by providing only temporary inhibition of gene function and unpredictable off-target effects. Recently, methods based on a bacterial CRISPR-associated protein-9 nuclease (Cas9) from Streptococcus pyogenes have generated considerable excitement. CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) and CRISPR-associated (Cas) genes are essential for adaptive immunity in select bacteria and archaea, enabling the organisms to respond to and eliminate invading genetic material.

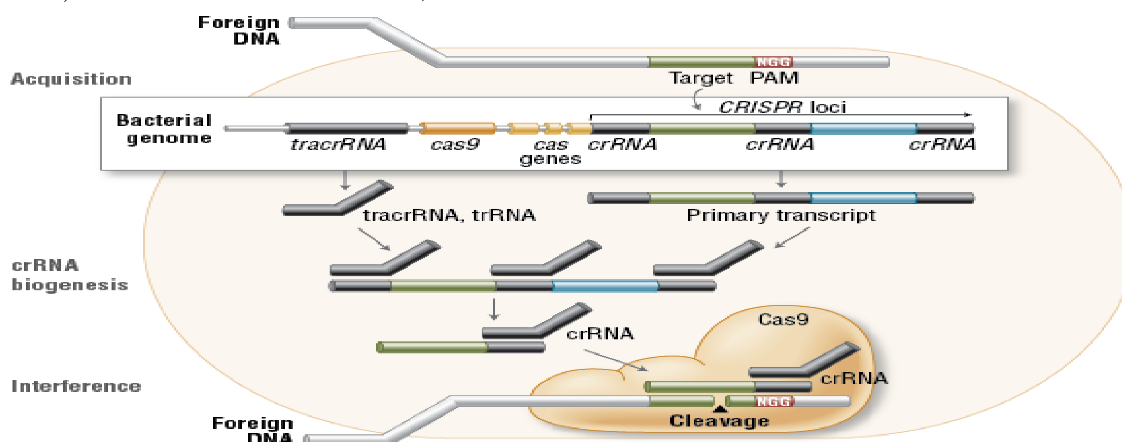


Fig. 2

The simplicity of the CRISPR nuclease system, with only three components (Cas9, crRNA and tracrRNA) makes this system attractive for laboratory use. By combining the crRNA and tracrRNA into a synthetic single guide RNA (sgRNA), a further simplified two-component system can be used to introduce targeted double-stranded breaks in genomic DNA. Breaks activate repair through error prone Non-Homologous End Joining (NHEJ) or Homology Directed Repair (HDR). In the presence of a donor template with homology to the targeted locus, the HDR pathway may operate, allowing for precise mutations to be made. In the absence of a template, NHEJ is activated, resulting in insertions and/or deletions (indels), which disrupt the target locus.

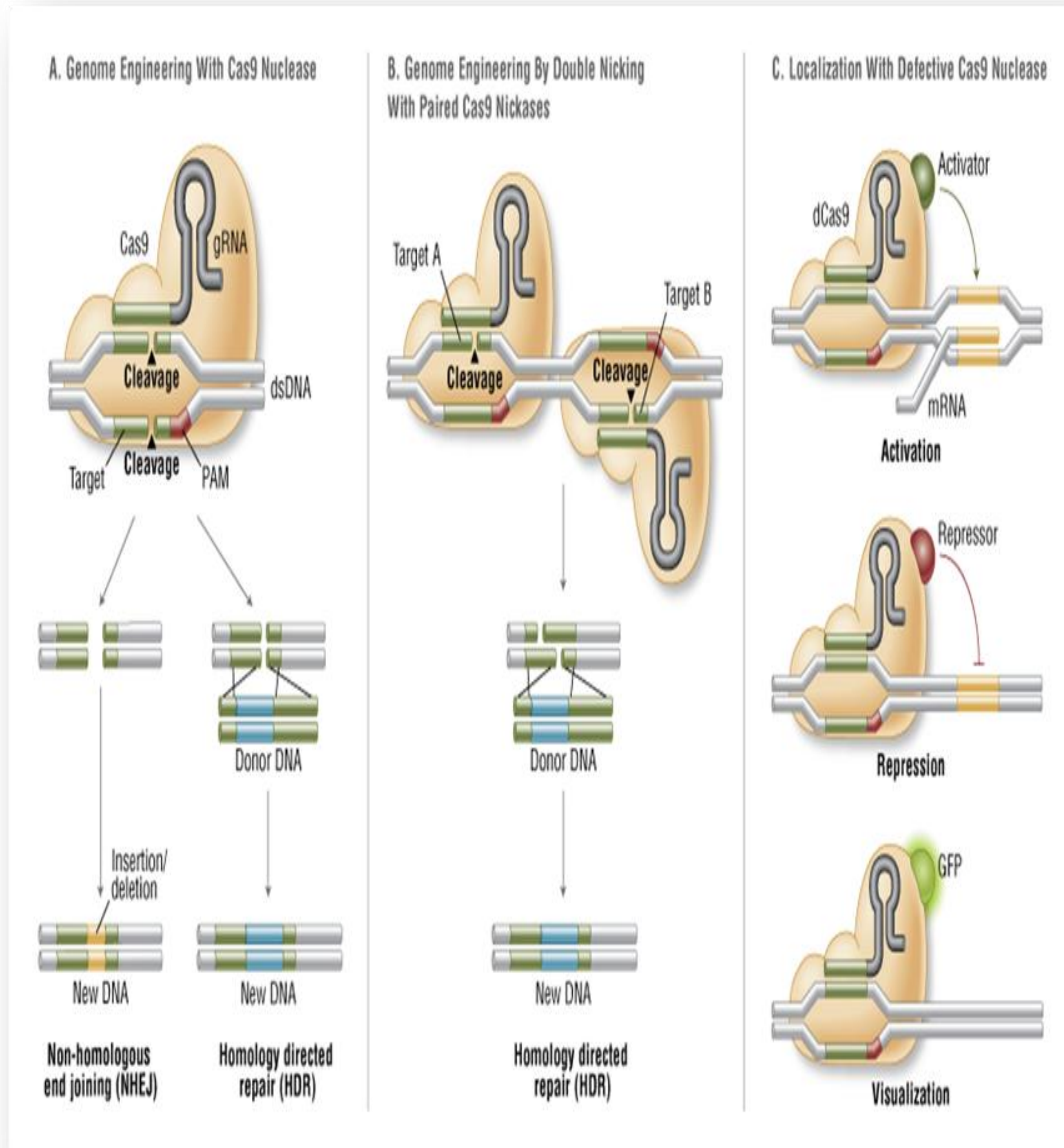


Fig. 3

Targeted mutagenesis using embryonic stem (ES) cells[13]:

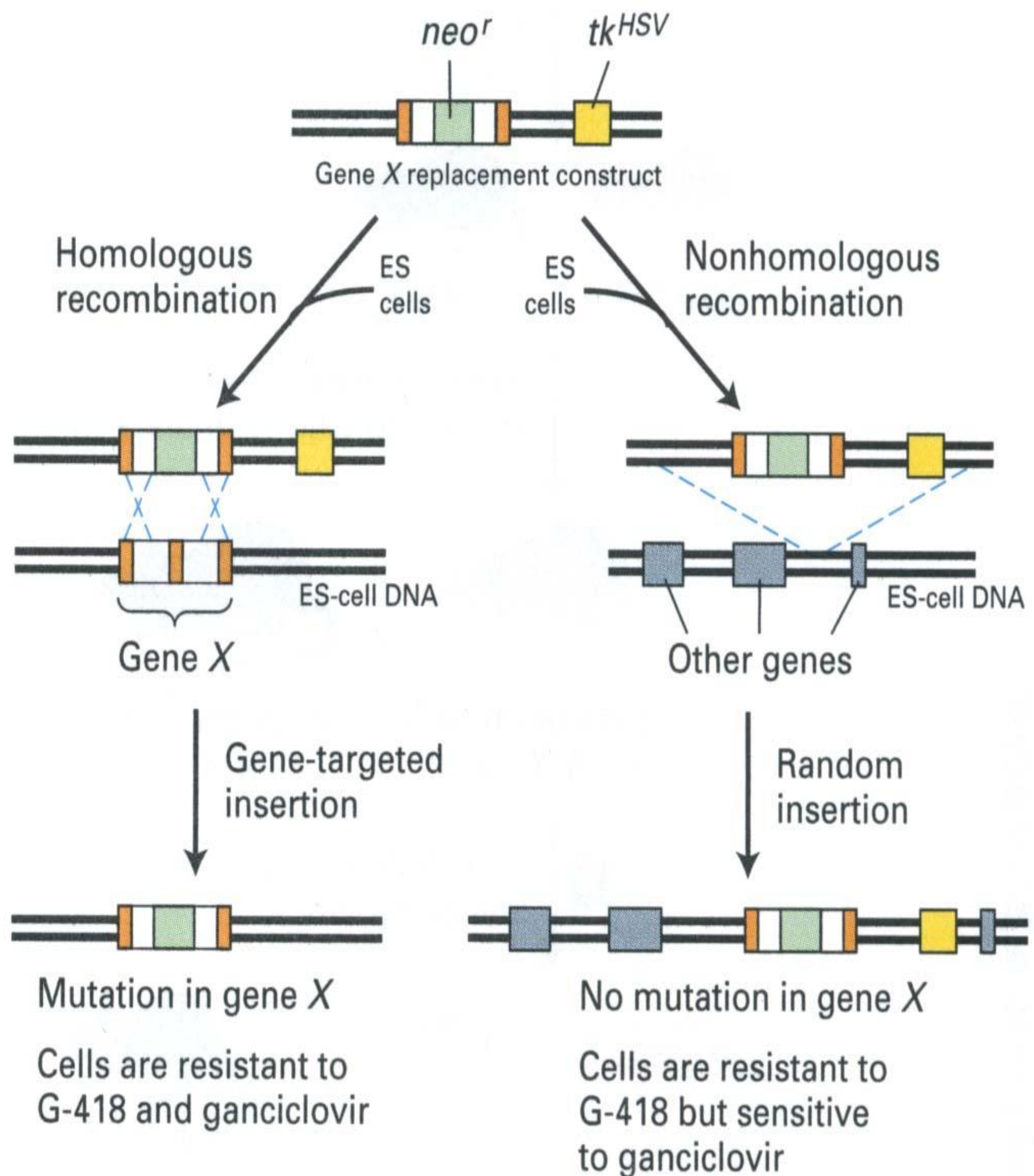
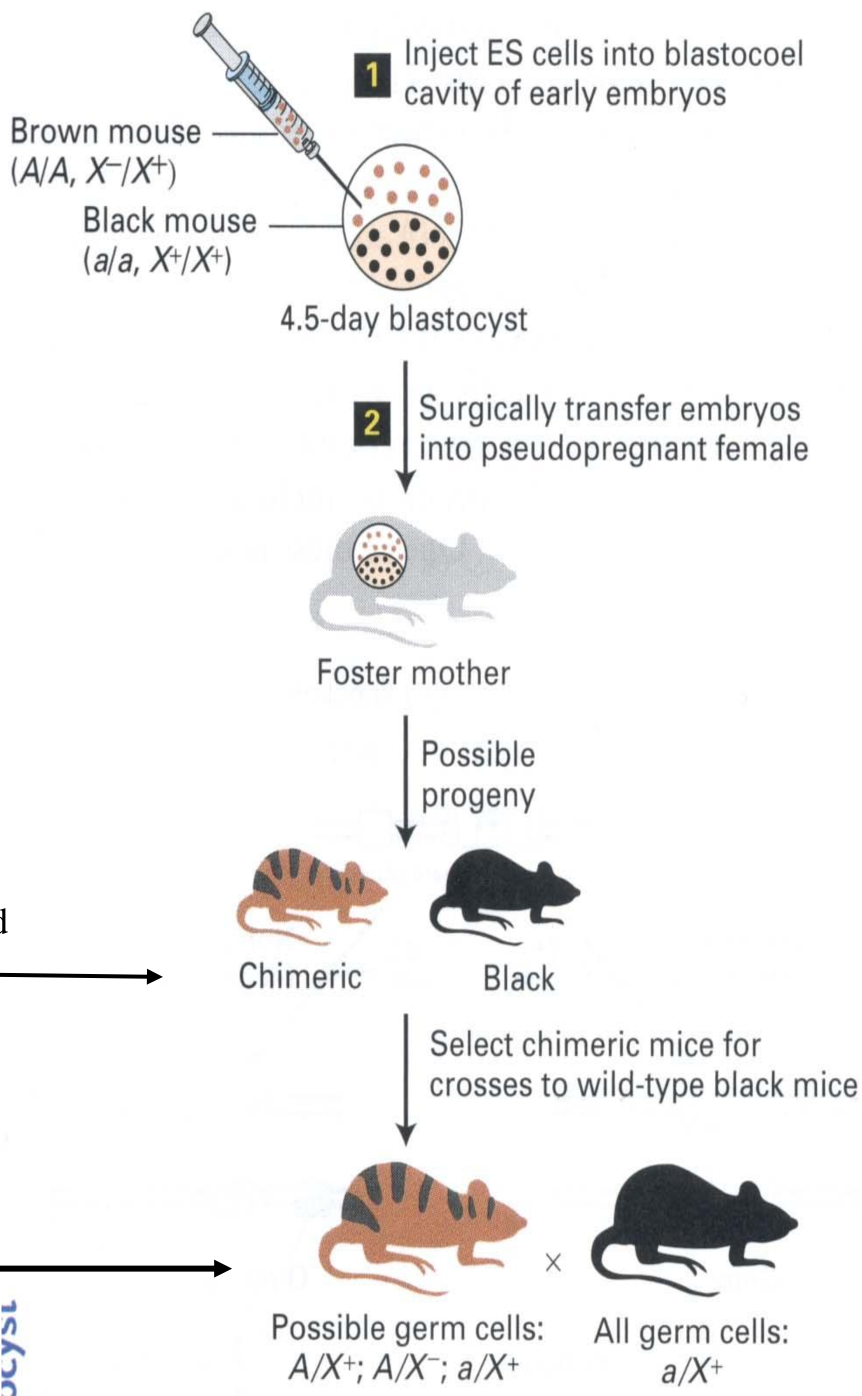


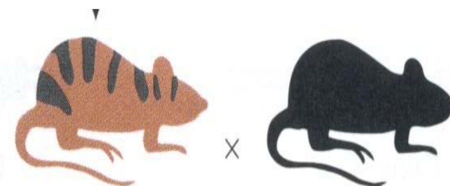
Fig . 4

ES cells heterozygous for a disrupted gene are used to produce homozygous 'knock outs'



Genetic chimeras are easily identified according to coat colour

If transgenic ES cells contribute to germ line, crossing chimeras to wt mice will result in Heterozygous off-spring.



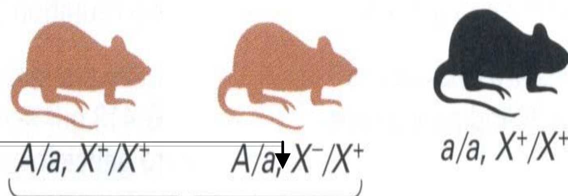
×

Possible germ cells: A/X^+; A/X^-; a/X^+

All germ cells: a/X^+

ES cells heterozygous for a disrupted gene are used to produce homozygous ‘knock outs’

3 ES cell-derived progeny will be brown



Only 50% of brown progeny will contain the transgene

A/a, X^+/X^+ A/a, X^-/X^+ a/a, X^+/X^+

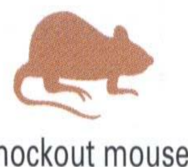
Progeny from ES cell-derived germ cells

4 Screen brown progeny DNA to identify X^-/X^+ heterozygotes

Molecular screening to identify X^-/X^+ heterozygotes

5 Mate X^-/X^+ heterozygotes

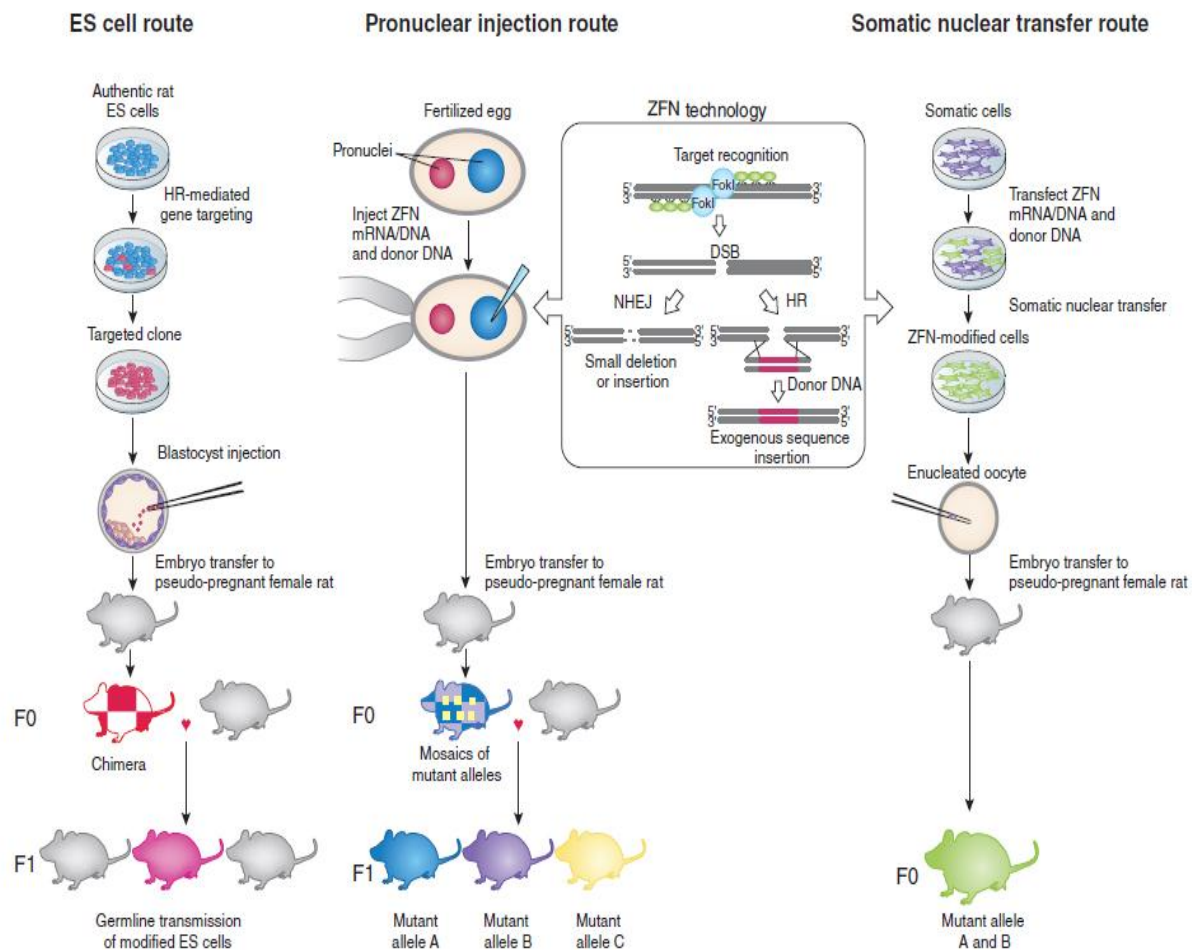
6 Screen progeny DNA to identify X^-/X^- homozygotes



Knockout mouse

Investigate phenotype

Targeted genome modification in mammalian cells

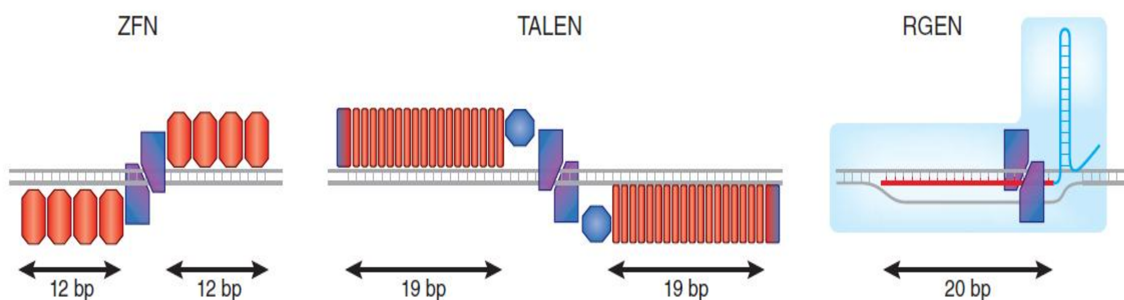


+
`

- Zinc Finger Nucleases (ZFNs)
- Transcription Activator-Like Effector Nucleases (TALENs)
- Type II clustered, regularly interspaced, short palindromic repeat system (CRISPR)

(provides prokaryotes with adaptive immunity to viruses and plasmids)

Technologies for achieving targeted gene modification[14]



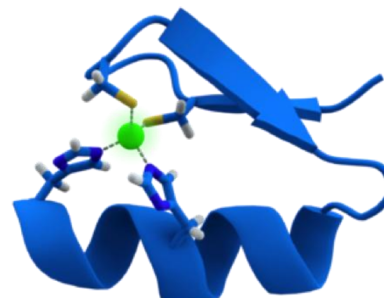
Site-specific modifications with meganucleases[15]

Nucleases induce site-specific double-strand breaks triggering:

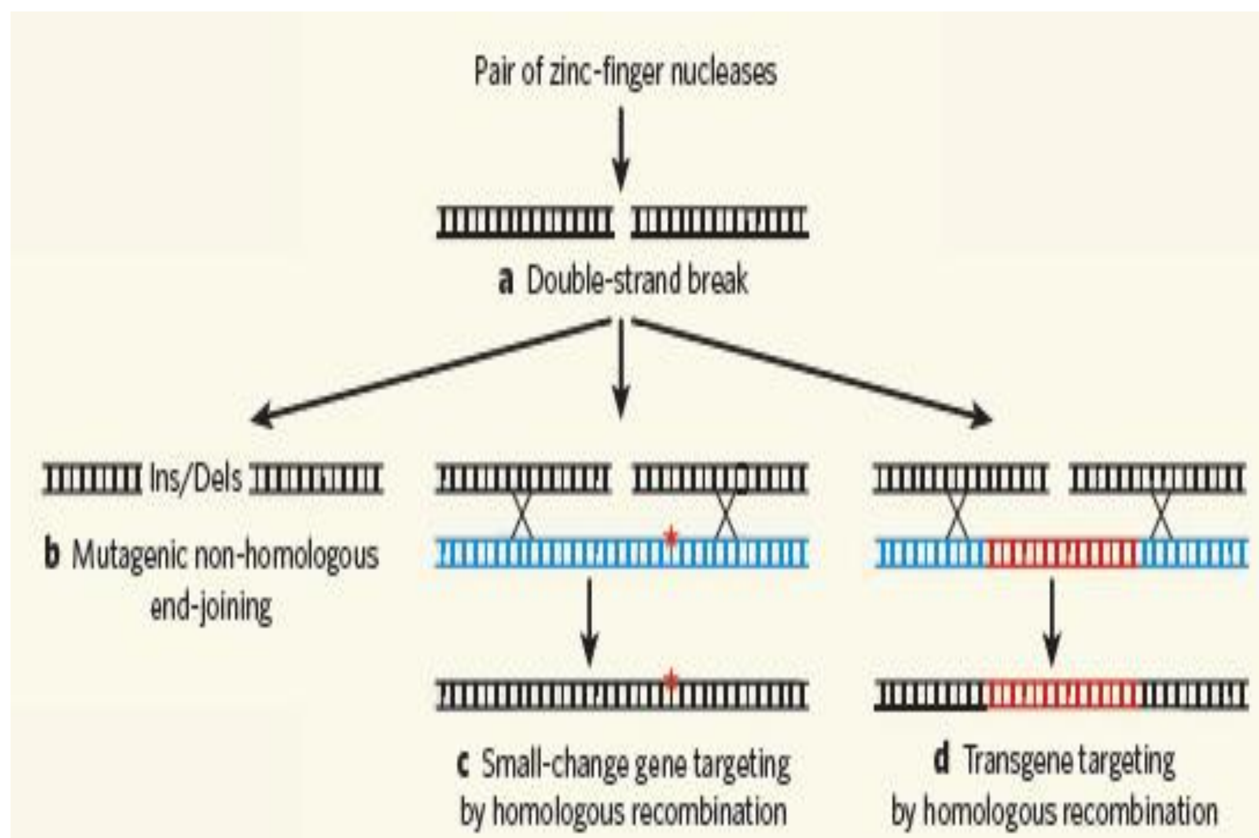
Mutagenic NHEJ

Small changes in target gene sequence (HR)

Gene replacement (HR)



Site-directed nucleases[16-26]:



Other Methods :

Fig. 5
Davis, D., &Stokoe, D. (2010). Zinc finger nucleases as tools to understand and treat human diseases. *BMC medicine*, *8*(1), 42.

All of the SDN techniques use the same basic mechanism of double strand breaks (DSBs).The nucleases are designed to recognize a specific DNA locus and cleave the DNA. The DSBs are naturally occurring deleterious DNA lesions and living organisms have developed mechanisms to repair them[17]. Non-homologous end joining (NHEJ) is the main pathway the cells use to repair DSBs and involves the exposed DNA ends being directly reconnected. Since NHEJ is error prone, the repair is often associated with insertions or deletions (together called indels) of one or more nucleotides[18,19]. If the

DSB creates overhangs (i.e. the two strands of DNA do not break at the same point), the NHEJ can also enable the introduction of a DNA template if the corresponding overhang on the other strand is compatible[20]. The second mechanism, homologous recombination (HR), is based on a template homologous to the sequence surrounding the DSB. The template is present in the chromosome in case of a naturally occurring HR; however, if an external template is delivered, the HR can be used to make custom changes to the genome including insertions of an exogenous DNA sequence[18,20,21]. Due to the provided template, the change made by HR is usually exact[21]; however, compared to NHEJ, it occurs much less often and various strategies have to be applied to increase the efficiency, e.g. overexpression of proteins or negative selection markers[10,20].

There are three ways of using SDNs that differ in the presence and/or type of the repair template[21,28,29]. In the case of SDN-1, no template is provided and the edition involves a random mutation of one or a few base pairs. In the case of SDN-2, the template provided is homologous but not identical to the target sequence and can introduce small specific changes to the sequence. In the case of SDN-3, a large DNA template is involved that could be an entire gene that may be cis-, intra-, or transgenic[21,29]. Since the recognition domains of the nucleases are very long (typically 18-40 bp), the techniques offer an unprecedented specificity and precision of the genome changes[22,18]. However, the techniques require a detailed knowledge of the structure of the chromosome and the function of the different genes, which is not available for all organisms and desired traits[22].

Moreover, even with the high specificity of the techniques, the nuclease might still cleave additional sites that are similar to the target sites potentially causing unwanted mutations, known as off-target effects, which is one of the issues of SDNs in general and should be carefully monitored[10,22,32]. Software tools are being developed to minimize the probability of off-target effects[23,33], however, the production of an organism with the desired mutation might still require a screening process to eliminate undesirable traits. Nonetheless, compared to conventional transgenesis or induced muta- genesis, the SDNs provide a much less laborious and more straightforward approach[24,28].

There are a number of reviews focusing on the principle of the different SDNs, see for example [10,18]. Here, we provide only a brief explanation of the SDNs and their possible applications.

- **Meganucleases**

MNs (also called homing or rare-cutting endonucleases) were the first of the SDNs used to produce genome manipulations via DSBs[25,10]. The MNs are naturally occurring endonucleases that identify specific DNA sequences and several hundreds of different MNs have been recognized so far in eukaryotes, bacteria and archea. The advantage of MNs is their small size, thus, making them suitable to a majority of delivery methods[18]. However, the a priori fixed target sites of the MNs are not common and the customization of the sites is very challenging and time-consuming due to the DNA-binding domain not being clearly separated from the catalytic domain, hence customization may compromise the enzymatic activity[16,18,34]. Therefore, the potential of MNs to be commonly used in genome editing is limited[25].

- **Zinc finger nucleases**

The ZFNs are composed of two independent regions: a recognition domain of zinc fingers each identifying a nucleotide triplet of the target DNA sequence and a non-specific nuclease, called FokI, creating theDSB[17,18]. Since the nuclease needs to dimerize in order to be active, the ZFNs are used in pairs. Like MNs, ZFNs are also relatively small and the design of the recognition domain is more straightforward compared to MNs by simply combining different zinc fingers[18]. However, ZFNs are more prone to off-target effects and have shown to have negative effects on cell proliferation[25,30].

- **Transcription activator-like effector nucleases**

Similarly to ZFNs, TALENs are composed of two independent parts. The first part originates from the transcription activator-like effectors (TALEs), a group of proteins first discovered in plant bacterial pathogens of the genus Xanthomonas[35]. During the bacterial infection, TALEs are transported directly into the plant cells where they bind to specific DNA sequences and modulate the expression of plant genes to promote the bacterial pathogenesis[16,25,10,18]. After the DNA binding codes of the natural TALEs have been identified, it is now possible to design artificial TALEs targeting any desired DNA sequence in both plants and animals[1018]. The TALEs are then fused with a FokI nuclease domain which again creates the DSB. Compared to ZFNs, the design is more straightforward, and longer recognition sites increase the specificity of TALENs making it less prone to off-target mutations and also less likely to cause deleterious effects[25,10]. When used in groups, TALENs allow multi- plexing, also called gene stacking (i.e. insertion of multiple genes at once). A slight disadvantage is the increased size which makes the delivery of TALENs to cells more challenging compared to ZFNs [22].

- **The CRISPR/Cas9 system**

The CRISPR/Cas9 system is part of the adaptive defence system in prokaryotes first discovered in E. coli[31,36]. It consists of guide RNAs that direct a

nuclease, e.g. Cas9, which is utilised by bacteria such as Streptococcus pyogenes in their adaptive immunity systems to recognize and cleave a specific site in the target DNA[25,18]. The CRISPR/Cas9 system is the most recent of the SDNs[37].The design is straightforward and can be accomplished quickly as the only part that needs redesign is the guide RNA[25]. The CRISPR/Cas9 system also allows multiplexing to generate organisms with multiple mutations or large chromosomal deletions[20] The CRISPR/Cas9 system and TALENs are the most promising SDNs.

Genome Editing in Plants[37-42] :

- **The importance of mutants in gene discovery**

The visible phenotypes of loss-of-function or gain- of-function mutants provide valuable clues as to the functions of genes of interest. For instance, an analysis of a set of growth-retarded dwarf mutants of the model plant Arabidopsis thaliana revealed both the metabolic and signal transduction pathways by which the plant steroid hormones, brassinosteroids (BRs), promote growth. However, mutants with defects in some enzymatic steps are elusive. In such cases, sequence-specific mutagenesis would be a useful approach for analysing gene function; however, in contrast to the situation in mice, yeast and Escherichia coli, homologous recombination- based mutagenesis techniques are not available for Arabidopsis. Thus, genetic studies in Arabidopsis involve random mutagenesis, followed by the identification of mutants with defects in a specific gene of interest.

- **Agrobacterium-mediated gene-tagging mutagenesis**

Feldmann and colleagues used Agrobacterium-mediated Transfer (T)-DNA insertional mutagenesis to randomly tag genes in Arabidopsis. The initial collection of Feldmann's T-DNA mutants led to the discovery of a host of genes involved in various physiological processes in Arabidopsis1, such as AGAMOUS (which functions in floral organdetermination), GLABRA1 (hair development), COP1 (light signalling), AUX1 (auxin transport) and HYPOCOTYL3 (phytochrome B signalling). To date, hundreds of thousands of T-DNA mutants have been generated in Arabidopsis, and the genomic DNA sequences flanking the T-DNA tags have been sequenced in efforts to map individual insertional events in the genome2. Multi-million dollar projects yielded T-DNA insertion mutants for over 80% of the ~28,000 genes present

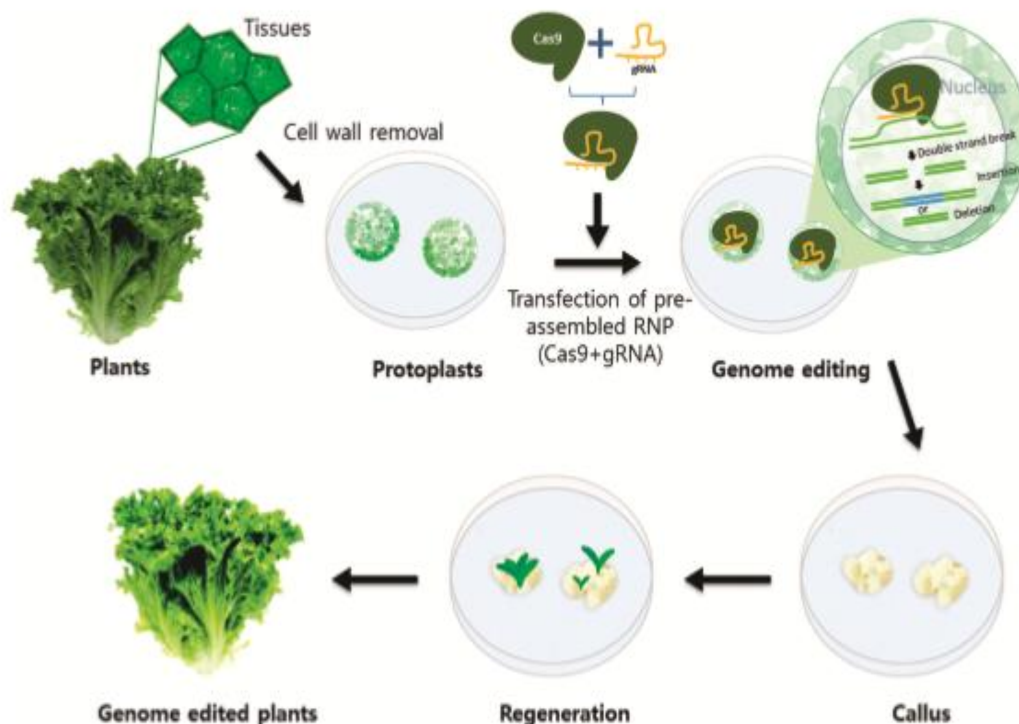


Fig6.
(Cas9 protein-based genome editing in plant cells.)

Protoplasts (cells lacking a cell wall) were prepared by treatment with cell wall-digesting enzymes. Cas9 protein and gRNA were independently prepared and assembled in vitro before being introduced into the protoplasts. The protoplasts divided after recovering their cell wall. Dividing cells formed callus (a mass of undifferentiated plant cells). Independent calli derived from a single protoplast were tested for successful genome editing by Polymerase Chain Reaction (PCR), Restriction Fragment Length Polymorphism (RFLP) and deep sequencing. Whole plants were regenerated from the mutation-bearing calli.

Despite the community-wide availability of an Arabidopsis T-DNA mutant population and extensive genetic analysis, more than 20,000 genes have no associated visible phenotype3. Many Arabidopsis genes exist as multiple and functionally redundant copies, and thus loss-of-function of any one of these genes does not result in a visible phenotype. For instance, 244 cytochrome P450 (CYP) genes4 and 694 F-box protein genes have been reported in Arabidopsis5, the majority of which await functional characterization. One approach to generate visible phenotypes for functionally redundant genes involves creating higher order mutants. However, this is time-consuming and is not always possible, especially when the genes of interest are closely linked on the same chromosome. Targeted mutagenesis for one or multiple genes is an elegant strategy to generate mutants for the thousands of genes with no associated T-DNA insertions, and higher order mutants for functionally redundant genes. Whilst this can sometimes be achieved by RNA interference (RNAi), this technology has limitations; genome editing offers a promising alternative.

Advantages [27]:

1. Used as a potent gene drive

Researchers have used the technique to disable retroviruses threatening organ transplantation from pigs to humans. It has also been used as a potent gene drive to allow rapid transmission of introduced genes throughout insect populations.

2. Easy Method

The beauty of genome editing is that without adding an extra piece of DNA, the genetic make-up can be altered, thereby avoiding the sobriquet “transgenic”. As such, leading regulatory authorities in the US and the EU have declared that genome-edited organisms are not transgenic organisms and, therefore, do not come under any biotech regulation. The first group of organisms that have escaped the regulatory dragnet are mushroom and maize in the US and canola in Germany.

3. Contributes significantly to biotechnology research

Imagine what people can do if they can transplant genes from one species to another. The possibilities are endless. In the field of biotechnology, genetic engineering paved the way for xenotransplantation or the process of transplanting living tissues or organs from animals to humans or vice versa. The research revealed the possibility of using pig organs as replacements for human hearts and kidneys, considering that they have similar physiology and size. It also led to tissue engineering that is now considered an alternative to replacement of cartilage, cerebrospinal shunts, heart valves and other organs. Suffice to say that plenty of things can be achieved with genome editing.

4. Increases the possibility of eradicating hunger

Among the many species and items that are genetically modified, plants are a favorite subject. Companies that want to create a sweeter tomato, bigger cherries and herbicide resistant crops can do so through GM. There may be health and safety concerns attached to the genetically engineered food and crops, but proponents assure that the breeding process is only an extension of the natural way. After all, the tissues used for the cell culture still come from a living organism.Because it is now possible to produce food and crops that are bigger and grow faster, resistant to disease, can thrive in different environments, or can be customized based on the soil composition and availability of water in a location, world hunger could be minimized if not completely eliminated. But there is still a question of what genetically modified crops can do to human bodies, their effects and long-term impact.

5. Saving endangered species

The same technology used to edit human genes can be used on animals. This could mean protecting a species like the Tasmanian devil, now endangered by an infectious cancer, or engineering the East Coast's chestnut trees to resist the chestnut blight that has devastated their growth.

“We’re faced with the sixth great mass extinction," Gary Roemer, a wildlife ecologist at New Mexico State University, said in an interview, "and this allows us to avert or perhaps just postpone the decline of certain species.”On the other hand, he and others were horrified at the possibility that someone might use gene editing as justification for putting off a species rescue “because we can always solve the problem later.”

Disadvantages [27]

1. Can disrupt the gene function in Human Embryos:

The potential to use CRISPR-Cas9 to disrupt gene function in human embryos is paralleled by technological breakthroughs that now allow us to measure global gene expression profiles at the single-cell level. Single-cell RNA sequencing has not only further identified differences between mouse and human, but has also provided a transcriptional atlas of human pre-implantation development that can now indicate which genes should be targeted for functional studies Furthermore,thistechnologywill further facilitate analysis after gene targeting as it can both identify cells carrying modified mRNA and simultaneously show the functional result at the global transcriptional level in successfully targeted cells.

2.Off-target effects:

The second issue is off-target effects, which are the consequence of the nonspecific activity of the Cas nuclease in non-target locations of the genome (Cho et al., 2014; Pattanayak et al., 2013. Even though off-target events might be scarce, they should not be overlooked, as there is the possibility that another gene could be mutatedcausinganeffectorphenotypethatcouldbeconfusedwith the one expected from the on-target mutation. As with mosaicism, off-targetevents pusses ofaproblemwhenworkingwithcultured cells than with embryos, as it is feasible to pre-select cell lines that carry the desired genotype without unwanted off-target mutations (Wuetal.,2015).Severalstrategiesarecurrentlyunderinvestigation to reduce the risk of off-target effects, such as improved algorithms to design gRNAsandengineeringCas9 enzymeswithhigherfidelity and specificity. In addition, this problem could be tackled by using multiple targeting strategiesagainst thesamegenetoensurethattheyproducethesame phenotype.

3. Has associated consequences and possible irreversible effects

Even scientists themselves believe that genetic engineering can have irreversible side effects, especially with hereditarily modified genes. After all, the process at the present uses viral factor to carry functional genes to the human body. Viral genes as they are, they are likely to leave certain side effects. Also, where the functional genes are placed in the genome is not exactly known. In the event that they replace other important genes instead of the mutated ones, other forms of diseases or health conditions are likely to develop. Is the world equipped to battle new illnesses that may turn out to be deadlier than ever?

4. Increased food supply can lead to adverse effects

Genetically modified wild rice is added with better carotene, which is needed by the human body to make vitamin A. This provides a perfect solution for vitamin A deficiency. Unfortunately, there are worries that GM organisms might actually be harmful to people. The added beta carotene levels aren't high enough to even make a difference as well. Herbicide resistant crops, on the other hand, may reduce the quantity of herbicide requirements, but it can lead to the growth of weeds that are resistant to herbicide and the loss of weed species that are essential to animal food and shelter. Suffice to say that modifying genes can have uncertain effects on humans and the environment.

5. There are risks in the method

In the case of transgenic biotechnology, blending animal and human DNA can have uncertain effects, including the creation of entities that possess degrees of intelligence or sentience atypical in non-human animals. Many also believe that there are health risks associated with genetically modified foods as well as in the experimental use of animals, long-term environmental impact, increased suffering of transgenic organisms, and possible creation of new diseases.

CONCLUSION:

The application of CRISPR-Cas technology to human cells has evolved in parallel with increasingly powerful methods of cell culture and analysis. It is now possible to modify the genome of a human embryo in a highly efficient and specific way, to grow theModifiedembryoin-vitroforlongerthanever before,andtoanalyze the regulatory consequences of the modification at the single cell level. With an increasing number of labs currently performing or abouttoembarkonthegeneeditingofhumanembryos, withinvision that that the use of CRISPR-based genetics, together with these technologicalbreakthrough,willdramaticallyaccelerateourefforts to decipher the mechanisms that control early human development. Although this in itself is a worthy pursuit, it is also hoped that such understanding will, ultimately, lead to improved infertility treatments and to the use of pluripotent stem cells in regenerative medicine.

REFERENCES:

1.Nature.com. Nature Publishing Group, n.d. Web. 25 Jan. 2015.

2.Nyren, P., The History of Pyrosequencing., Methods Mol Biol., 2006; 373:1-14

3. "Genome Sequencing: A History." Genome Sequencing: A History. N.p., n.d. Web. 25 Jan. 2015.

4."Genome Editing Timeline." EpiGenie Epigenetics and NonCoding RNA News.N.p.,n.d.

Web. 25 Jan. 2015.
5."How We Study The Microbiome." How We Study The Microbiome. N.p., n.d. Web. 23 Jan.2015.
6."What Is Zinc Finger Nuclease (ZFN) Technology?" Sigma-Aldrich.N.p., n.d. Web. 25 Jan. 2015.
7. www.genome-engineering.org
8. www.horizondiscovery.com
9. Jinek, M., et al. (2012) Science, 337, 816–821.
10. Overballe-Petersen, S., et al. (2013) Proc. Natl. Acad. Sci. U.S.A., 110, 19860–19865.
11. Gong, C., et al. (2005) Nat. Struct. Mol. Biol., 12, 304–312
12. Lodish H, Berk A, Matsudaira P. Molecular cell biology. (2004) Molecular genetic techniques and genomics. Chap. 9 p. 351-403. New York: W.H. Freeman and Company
13.Capecchi, M. R. (2005). Gene targeting in mice: functional analysis of the mammalian genome for the twenty-first century. Nature Reviews Genetics, 6(6), 507-5124.
14. Davis, D., &Stokoe, D. (2010). Zinc finger nucleases as tools to understand and treat human diseases. BMC medicine, 8(1), 42.
15. Mussolino, C., &Cathomen, T. (2013). RNA guides genome engineering. Nature biotechnology, 31(3), 208-209.
16. Curtin, S.J., Voytas, D.F., and Stupar, R.M. (2012). Genome Engineering of Crops with Designer Nucleases. Plant Genome 5, 42-50.
17. Harrison, M.M., Jenkins, B.V., O'Connor-Giles, K.M., and Wildonger, J. (2014). A CRISPR view of development. Genes Dev. 28 , 1859-1872.
18. Rinaldo, A., and Ayliffe, M. (2015). Gene targeting and editing in crop plants: a new era of precision opportunities. Mol. Breed. 35 , 1-15.
19. Bortesi, L., and Fischer, R. (2015). The CRISPR/ Cas9 system for plant genome editing and beyond. Biotechnology Advances 33, 41-52.
20. Belhaj, K., Chaparro-Garcia, A., Kamoun, S., and Nekrasov, V. (2013). Plant genome editing made easy: targeted mutagenesis in model and crop plants using the CRISPR/Cas system. Plant Methods 9, 39.
21. Podevin, N., Devos, Y., Davies, H.V., and Nielsen, K.M. (2012). Transgenic or not? No simple answer! EMBO Rep. 13, 1057-1061.
22. Fichtner, F., UrreaCastellanos, R., and Ülker, B. (2014). Precision genetic modifications: a new era in molecular biology and crop improvement. Planta 239, 921-939.
23. Naito, Y., Hino, K., Bono, H., and Ui-Tei, K. (2014). CRISPRdirect: software for designing CRISPR/Cas guide RNA with reduced off- target sites. Bioinformatics 31, 1120-1123.
24. Lusser, M., and Davies, H.V. (2013). Comparative regulatory approaches for groups of new plant breeding techniques. N. Biotechnol. 30, 437-446.
25. Puchta, H., and Fauser, F. (2014). Synthetic nucleases for genome engineering in plants: prospects for a bright future. Plant J. 78 , 727-741.
26. http://greengarageblog.org/
28. Araki, M., and Ishii, T. (2015). Towards social acceptance of plant breeding by genome editing. Trends Plant Sci. 20, 145-149
29.Hartung, F., and Schiemann, J. (2014). Precise plant breeding using new genome editing techniques: opportunities, safety and regulation in the EU. Plant J. 78, 742-752.
30.Ousterout, D.G., Kabadi, A.M., Thakore, P.I., Perez-Pinera, P., Brown, M.T., Majoros, W.H.,Reddy, T.E., and Gersbach, C.A. (2015). Correction ofDystrophin Expression in Cells FromDuchenne Muscular Dystrophy Patients Through Genomic Excision of Exon 51 by Zinc Finger Nucleases. Mol. Ther. 23, 523-532.
31.Ishino, Y., Shinagawa, H., Makino, K., Amemura, M., and Nakata, A. (1987). Nucleotide sequence of the iap gene, responsible for alkaline phosphatase isozyme conversion in Escherichia coli, and identification of the gene product. J. Bacteriol. 169, 5429-5433.
32.Carlson, D.F., Tan, W., Hackett, P.B., and Fahrenkrug, S.C. (2013). Editing livestock genomes with site-specific nucleases.Reprod. Fertil. Dev. 26, 74-82.
33.Xie, S., Shen, B., Zhang, C., Huang, X., and Zhang, Y. (2014). sgRNAcas9: A Software Package for Designing CRISPR sgRNA and Evaluating Potential Off-Target Cleavage Sites. PLoS ONE 9,e100448.
34.Liu, Z., Zhao, Y., Li, X., Song, J., Yang, Y., et al. (2013). Highly Efficient Generation of GGTA1 Biallelic Knockout Inbred Mini-Pigs with TALENs. PLoS ONE 8, e84250.
35.Bonas, U., Stall, R.E., and Staskawicz, B. (1989). Genetic and structural characterization of the avirulence gene avrBs3 from Xanthomonascampestrispv.vesicatoria. Mol. Gen. Genet. 218, 127- 136.
36.Jansen, R., Embden, J.D.A.v., Gaastra, W., and Schouls, L.M. (2002). Identification of genes that are associated with DNA repeats in prokaryotes. Mol. Microbiol. 43, 1565-1575.
37.Boettcher, M., and McManus, Michael T. (2015). Choosing the Right Tool for the Job: RNAi, TALEN, or CRISPR. Mol. Cell 58, 575-585.