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Targeted pinpoint gene editing tool, CRISPR/Cas9: A Review

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Abstract

CRISPR has emanated as a powerful tool for targeted, precision genome editing and is extensively captivating biomedical research world nowadays. Being more precise, faster and cheaper than predecessor DNA editing strategies like ZFN (Zinc Finger Nucleases) and TALENs (Transcription activator-like effector nucleases), the horizon of its potential application has been extremely widened. In this technique, bacterial machinery is being used to study and treat various human diseases, having gene-based etiology, β Thalassemia, spinal muscular dystrophy, cystic fibrosis and microcephaly. Additionally, CRISPR/Cas9 has also been applied in studying immune diseases e.g. AIDS. Moreover, its use in enhancing genetic code of crops and livestock with large-scale production of biomedical materials, is also gaining much glamor. Unlike somatic cells, the use of CRISPR/Cas9 in gene manipulation of germline cells is controversial. Due to anticipated and existing ethical ramifications, it would probably take a few more years to routinely use CRISPR/Cas9 in humans. This review has been done to explore different aspects of CRISPR/Cas9, including its current and future implications.

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Introduction

CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) is captivating the entire scientific world which is a powerful revolutionary genome editing tool adapted from immune or self-defense system of bacteria/archaea (Horvath and Barrangou, 2010; Cong *et al.*, 2013). When a virus attacks, bacterium saves DNA snippets of the attacking virus and use them against subsequent attacks by the same or related viruses; Keeping the DNA record of the invader viruses, helps bacterium and its offspring in disabling the future invaders. This forms the basis of CRISPR/Cas9-based targeted precision gene editing in various organisms, such as: baker's yeast or *Saccharomyces cerevisiae* (DiCarlo *et al.* 2013; Zhang *et al.* 2014; *Liu et al.* 2016), zebra fish(Hwang *et al.* 2013), *Drosophila melanogaster* (Gratz *et al.* 2013), nematodes (Friedl and *et al.* 2013),plants (Jiang *et al.* 2013), mice (Wang *et al.* 2013), monkeys (Guo and Li 2015) and humans (Baltimore *et al.* 2015).

CRISPRs (a family of DNA segments in bacteria) are specialized segments of DNA with two distinct features: nucleotide repeats and spacers. These palindromic nucleotide repeats are spread throughout the entire CRISPR region while, spacers (DNA fragments derived from viruses and plasmids) lie interspersed among them (Bolotin *et al.* 2005; Mojica *et al.* 2005; Pourcel *et al.* 2005; Marraffini and Sontheimer 2010). Within CRISPR array, next to repeat-spacer section, there lie tiny clusters of *cas* or CRISPR-associated system genes. The third major component of CRISPR locus is a leader sequence, which is A-T rich (Hille and Charpentier 2016) Arrangement of three components: *cas* genes, repeat-spacer array and leader sequence is variable (Horvath and Barrangou 2010; Marraffini and Sontheimer 2010) (Fig. 1).

Another essential component of CRISPR/Cas system is Cas9 (CRISPR-associated system) enzyme, an endonuclease which in its active form assists in modifying DNA. It cuts the double stranded DNA, allowing an easy removal or addition of desired DNA bits. Different variants of Cas9 have been discovered, each having different function, depending upon the DNA recognition site. Cas9 has two domains for binding the two different RNAs: crRNA (CRISPR RNAs) and tracr RNA (trans-activating crRNA), which guide Cas9 to target site and make the desired cut. There are many different CRISPR/Cas systems, depending upon the nature of basic components and source organism. On the basis of the data sequenced, the researchers have categorized CRISPR/Cas systems into 2 Classes; 6 system-types (Wright *et al.* 2016) and, 19 subtypes (Westra *et al.* 2016). The simplest system among them CRISPR/Cas9 has been customized to manipulate genomes. Modified system includes a guide RNA (self-synthesized substitute of crRNA and Cas9) and Cas9, which are then delivered into the cell whose genome has to be altered (Hendel *et al.* 2015; Ledford 2015). For genome editing, a DNA repair template is also used by the CRISPR/Cas9 system. The aim of present study was to review and explore various aspects of CRISPR tool, its current status and future expectations, and to abridge the huge amount of information available about CRISPR into a single source.

Methods

Literature survey and selection criteria

Google web browser and Google scholar have been used for data mining. Latest research papers have been consulted to materialize this article. Data associated with different features, current aspects and future perspectives about CRISPR/Cas technology has been considered.

Discussion/review findings

CRISPR/Cas9 working mechanism

The most studied, simplest mechanism involves type II CRISPR/Cas system. Simpler version of what this system actually does is, finding; cutting and pasting. The mechanism of action of CRISPR/Cas is a natural defense mechanism of bacteria, constituting three phases; acquisition, biogenesis and interference. During the acquisition phase (spacer acquisition), when a virus invades, bacterium cuts its DNA and incorporates it into CRISPR locus as a spacer.

Table 1. Comparison of CRISPR-Cas system with other gene editing technologies.

CRISPR-Cas System	ZFN, TALENs and Mega-nucleases
Low cost/affordable	High cost
Fast	Increased time consumption
High reliability	Less reliability
Versatility	No versatility
High specificity	Low specificity
Widespread use	No widespread use
Large scale studies	No Large scale studies
High-throughput studies	No High-throughput studies
Multiplex gene alterations	No Multiplex gene alterations
Flexibility for target selection	No Flexibility for target selection

In biogenesis, the CRISPR loci then undergo transcription; resultant transcripts are processed to produce crRNAs. crRNAs are then used to guide cas about invading target DNA on the basis of sequence complementarity. Then comes the interference, where Cas9 armed with a crRNA and tracr RNA, cuts the foreign DNA having a 20bp sequence, complementary to crRNA and lying in close vicinity of the PAM sequence (Protospacer Associated Motif: a conserved short sequence of 2 to 5 bp).

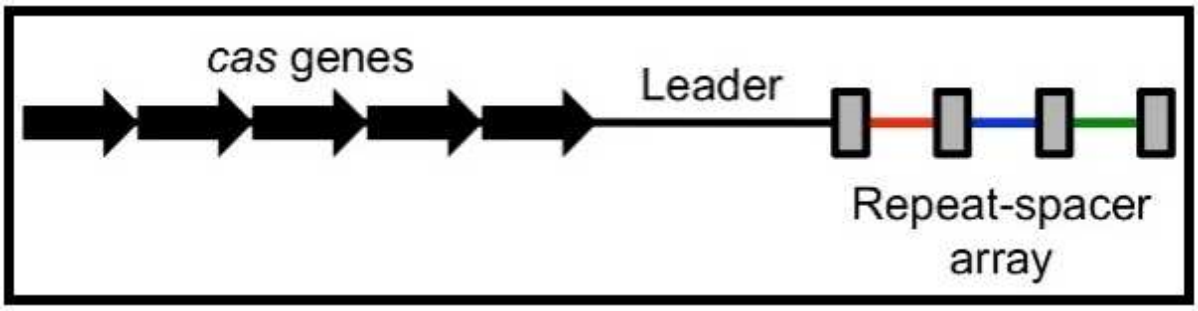

Fig. 1. CRISPR Locus (June 2011).

The tracr RNA usually has some complementarity with crRNA and is needed for its maturation. A combination of tracr RNA and minimum one crRNA is called Single guide RNAs or sgRNA. During this interference, the both nuclease domains (HNH and RuvC like) of Cas9 cut both strands (complementary and non complementary) of target DNA and, generate double-stranded breaks or DSBs (Fig. 2.).

When performing CRISPR experiment, researcher provides Cas9 with artificial gRNA (guide RNA), which directs it to the target DNA sequence. As Cas9 cuts the target sequence, cell tries to repair it by using the material it already has or with the template that we inject. Injected DNA then takes the place of targeted DNA, a change is thereby produced within the target gene. The more recent versions of CRISPR don't generate a cut rather, they have been provided with an ability to deliver Cas to a desired location within the gene and change the nucleotide bases e.g., A>G or C >T. Scientists use both viral (based on lentivirus, adenovirus and adeno-associated virus) and non-viral delivery systems for delivering Cas9 and sgRNA into the target cell, such as plasmids through electroporation, depending upon the cell type. A modern CRISPR system can cut 5-62 genes simultaneously (Pennisi 2013).

CRISPR/Cas9 based genome editing-Applications

Generating a Knockout

CRISPR tool can be used to produce knockout cells. This procedure requires two basic things: a genomic

target sequence and co-expression of one endonuclease (either Cas9 or its any substitute) and a gRNA; specific to this target sequence. The DNA sequence should be approximately equal to 20 bp, unique and lying in immediate vicinity of PAM (Protospacer Adjacent Motif), for high binding efficiency. Post expression Cas and gRNA form a ribo nucleoprotein complex, which binds the DNA target, having significant homology with gRNA spacer sequence. Cas9 then cleaves the target DNA, resulting in DSB or double-strand break within the target sequence.

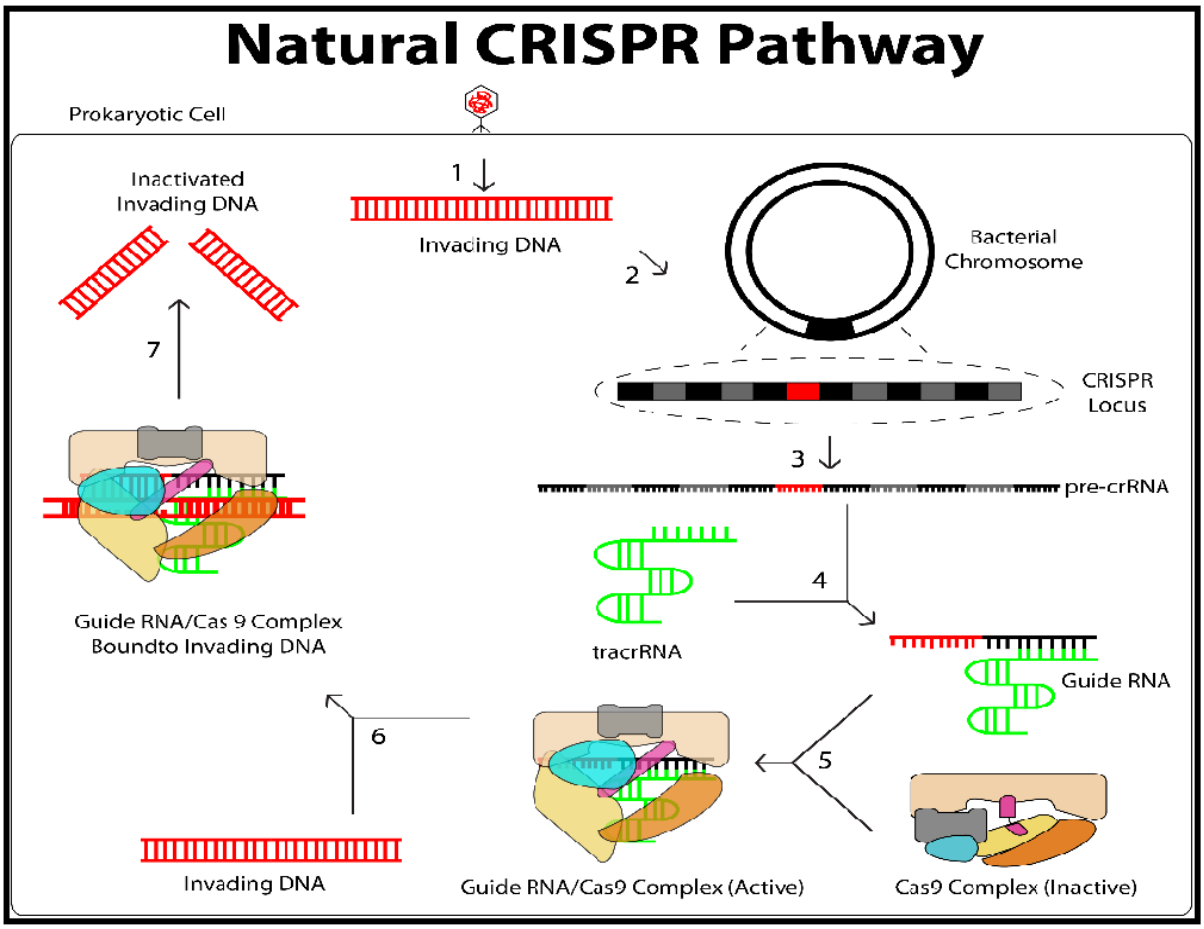

Fig. 2. Natural CRISR pathway. 1: DNA Invasion; 2: Incorporation of Invaded DNA into CRISPR array; 3: Pre-crRNA Transcription; 4: Guide RNA Formation; 5: Cas9 Activation; 6: Target Binding; 7: Target Cleavage. (Tufts.edu 2014).

There are two basic ways by which this DSB is repaired; either NHEJ/Non-Homologous End Joining pathway or HDR/ Homology Directed Repair pathway. NHEJ is more efficient but it more prone to errors as well while, HDR is less efficient but more reliable; With both ultimately resulting in an in-activating mutation within the gene of interest (Figure 3.).

Enhancing specificity with nickases and high-fidelity enzymes

The specificity of CRISPR relies partially upon the specificity of gRNA for the genomic target and also partially upon Cas9; If a researcher aims for high CRISPR specificity, he needs to optimize gRNA design and modify Cas9 too. Ideally gRNA designed, should not have any off-target site, as explained below. Cas9 should be modified in such a way that when off-targets exist, which in reality do, it performs less editing at those off-target regions. A wildtype Cas9 produces DSBs whereas, a D10A mutant SpCas9 produces NICK or single strand break; Therefore, two D10A nickases are needed for DSP generation within the target site.

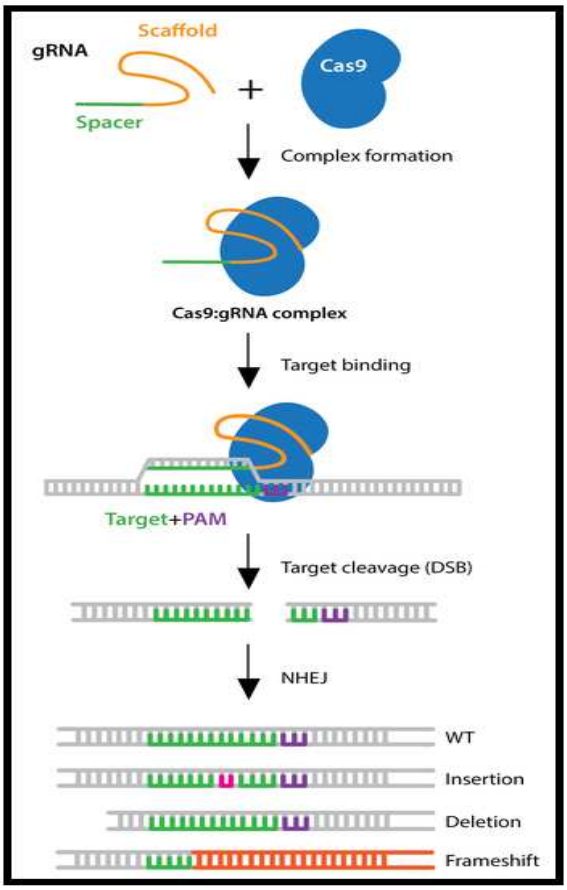

Fig. 3. Generating a knockout (Guide 2018).

This requirement vividly increases target specificity, since, it is unlikely that two off-target nicks will be generated within close enough proximity to cause a DSB. Three Cas9 enzymes possessing high fidelity: eSpCas9, SpCas9-HF1 and HypaCas9 have also been recently developed and being utilized for precision targeted editing (Fig. 4.).

Precise modification with HDR

Using HDR technique involves three things: a repair template (desired DNA sequence or edit to be inserted), which can be a single stranded or a double stranded oligonucleotide or even a DNA plasmid, having left and right homology arms (additional immediate homologous sequences); gRNA; Cas9 or nickase.

Repair template must lack the PAM sequence as the one existing in the genomic sequence, to enhance specificity (Fig. 5.). HDR with repairing results in alterations ranging from single base change to big insertions (tag, fluorophore).

Editing without DSBs

HDR repairing technique is less efficient therefore, scientists have generated novel CRISPR base editors for the production of point mutations without HDR. These base editors, as the name indicates, fuse Cas9 nickase to a cytidine deaminase and, convert cytidine to uridine. Additionally, some base editors have been developed which convert adenosine to inosine, producing a point mutation or base change (Fig. 6).

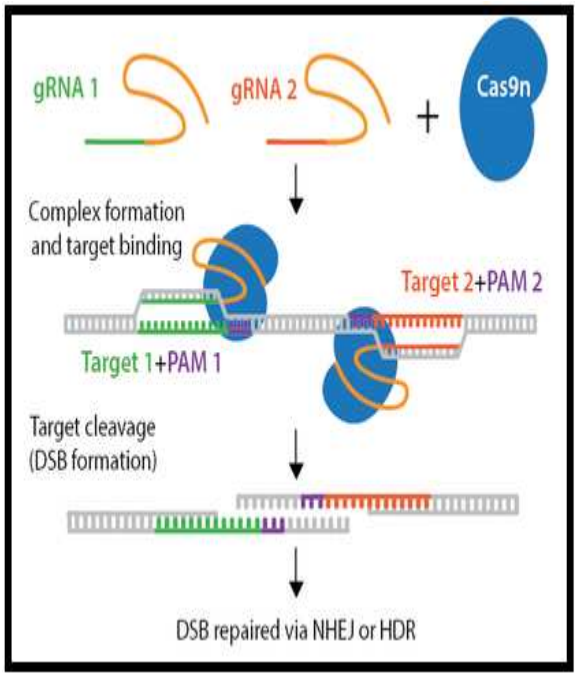

Fig. 4. Enhancing specificity with nickases and high-fidelity enzymes (Guide 2018).

Activations/Repression of target genes

Point mutations such as, D10A or H840A within SpCas9 result in a dCas9, which is a nuclease-dead Cas9, lacking the ability of target DNA cleave. But, thanks to gRNA-targeting sequence, this dCas9 still holds the ability of binding to the genomic target of interest. dCas9 has a power to repress the repress gene transcription, as it blocks initiation, when specifically targeted to the transcription start sites. Moreover, tagging dCas9 with transcriptional repressors/activators and then targeting them to the promoter site, causes huge transcriptional repression/activation of the downstream target-genes. The repressor complex therefore constitutes dCas9-based fused with a transcriptional activator and activator complex is the dCas9 fused with a transcriptional repressor.

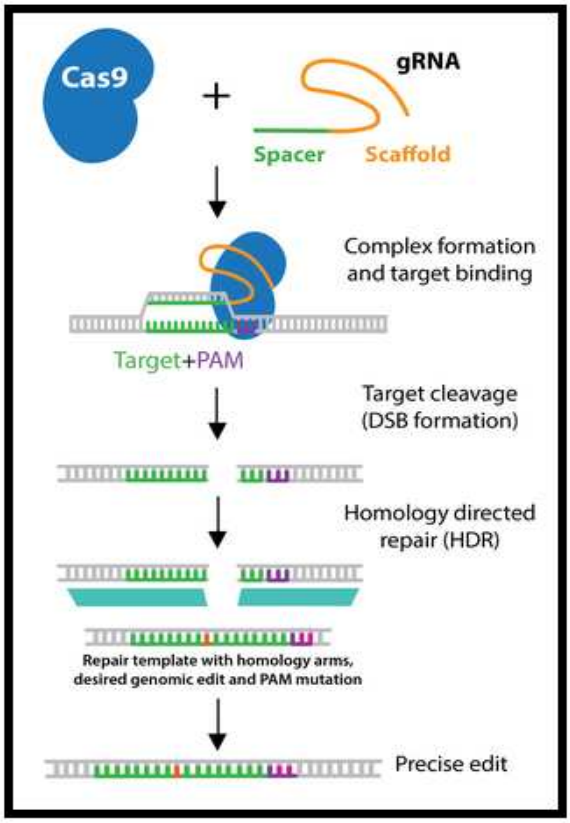

Fig. 5. Precise modification with HDR (Guide 2018).

Activation and repression are reversible effects, not permanent genomic DNA modifications (Fig.7.).

Epigenetic modifications

Fusing Cas endonucleases with epigenetic modifiers such as TET1 (Ten-eleven translocation methyl-cytosine dioxygenase 1) can help in programmable epigenome-engineering. Such combined gene editing tools do not produce DSBs and are highly specific However, they are much more specific for particular chromatin and DNA modifications, permitting the isolation of even effects caused by a single epigenetic-mark.

The tools, unlike repressors and activators, are persistent and inherited by the daughter cells (Fig. 8.).

Multiplex Genome Engineering

Multiplex CRISPR system application involves, causing multiple gRNAs to express from the same plasmid thereby, ensuring that each host cell within which the plasmid has been inserted, expresses all of required gRNAs.

In doing so one increases the likelihood of all desired genomic manipulations being carried out by Cas9, i.e. modification of multiple genes simultaneously.

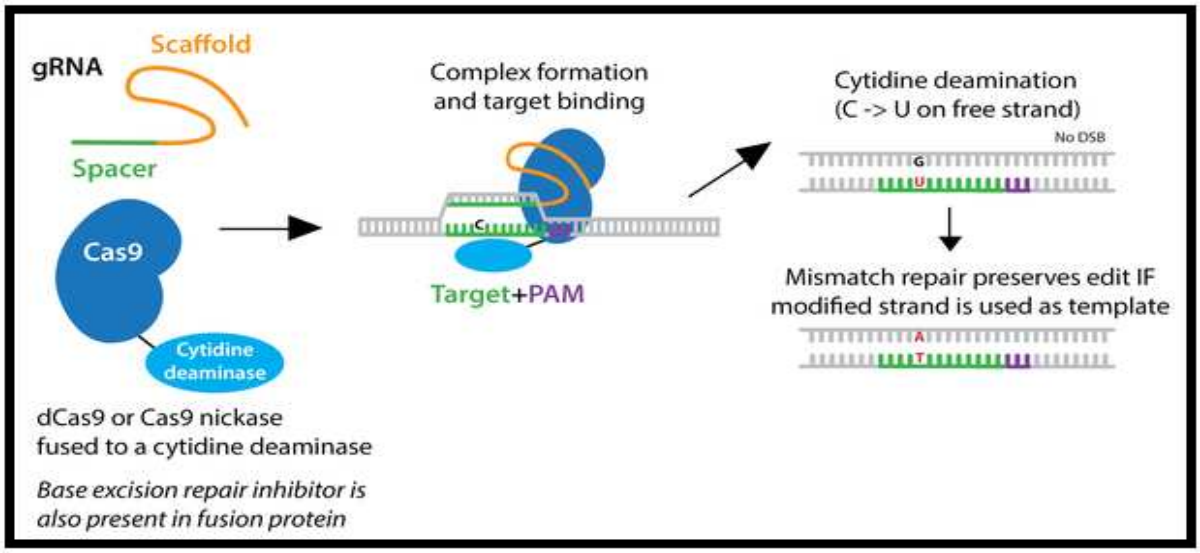

Fig. 6. Editing without DSBs (Guide 2018).

Genome-Wide Screens and CRISPR Pooled Libraries

A diverse population of lentiviral transfer vectors called Pooled lentiviral CRISPR libraries or CRISPR libraries) are currently, used for conducting genome-wide screens. Each vector contains specific gRNA r which is generated *in silico*. CRISPR libraries have been designed for common CRISPR applications, i.e.

g knockout, activation, and repression within mouse and human genomes.

The genome-wide screens employing CRISPR libraries provide a way of gathering an unbiased information about, which genes are causing particular phenotype/disease. Additionally, the gRNAs identified in a screen can be tested individually to confirm that they are causing the disease or desired phenotype (Fig.9).

Visualization of genomic loci using fluorophores

Fusion of catalytically-inactive Cas9 or dCas9 with any fluorescent marker such as GFP, makes dCas9 a DNA labeler that can be customized and has compatibility with fluorescence microscopy within the living cells (Fig. 10.).

Purification of genomic regions with dCas9

In order to purify any particular genomic sequence having a specific gRNA, scientists have extended ChIP (chromatin immunoprecipitation) by using CRISPR. enChIP (engineered DNA-binding molecule-mediated ChIP), an extended version has dCas9 which purifies genomic DNA which is bound by the gRNA.

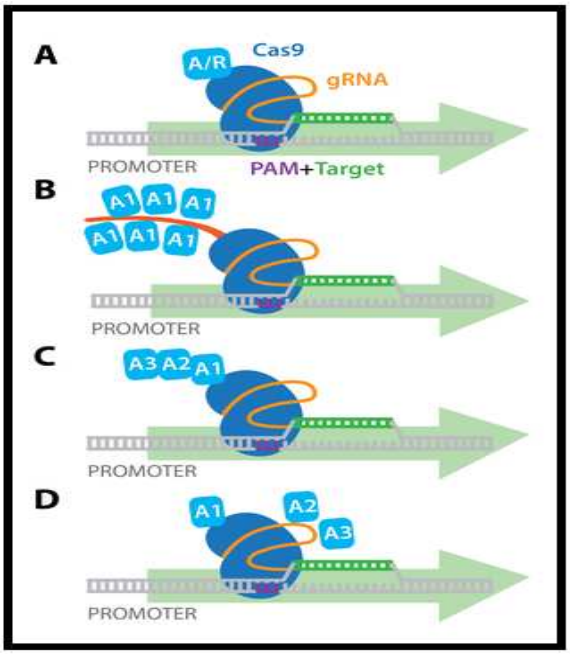

Fig. 7. Activations/Repression of target genes (Guide 2018)

An epitope tagging can be applied to dCas9 or even gRNA for better purification. Tags include: 3xFLAG-tag, biotin tags and PA. Post purification, locus identification is done by mass spectrometry, RNA-sequencing and next-generation sequencing (Fig. 11.).

RNA Targeting

Causing a mutation in the catalytic domain of Cas13, makes it a good RNA-binding protein. Cas13 occupies type VI CRISPR system and recognizes ssRNA (Single Stranded RNA) than recognizing dsDNA (Double Stranded DNA); Like other type VI nucleases Cas13, causes degradation of target RNA, once it is recognized by crRNA (Fig. 12.).

Planning a CRISPR Experiment

For researchers, genome manipulation has never been this powerful before, as it is with CRISPR/Cas9. Modification experiments with CRISPR/Cas9 gene editing tool have been done in variety of organisms. Such experimentation requires an organized framework, as the one described below (Fig. 13.).

Selection of cell line/organism and genomic sequence

To begin experiment one must select the organism or cell line in which manipulation is desired. The wise way of initiating the experiment is to sequence the region of interest before selecting CRISPR components. In any organism the efficiency observed would depend upon the number of alleles of target gene, which is variable from one cell line to another.

Selection of desired genetic manipulation

Once you have selected the cell line and the gene you want to manipulate in order to demonstrate the process/disease of interest, the very next step is selection of genetic manipulation process. There are four basic types of genetic manipulation:

Knockout: Permanent disruption of gene expression/function.

Edit: Sequence change, particularly made by user-defined point mutation generation or tag insertion.

Repress/interfere: Reduction of target gene expression, with no permanent genome modification.

Activate: Elevation of expression of endogenous target gene, with no permanent genome modification. Choice of genetic manipulation defines the reagents and CRISPR components required. Different reagents are required for carrying out genetic manipulation experiment in different organisms; Attention must be paid over the availability of desired reagents prior to begin navigation onwards. When one or more reagents for use in model organism is not available, customization of available materials can be made to suit the requirements.

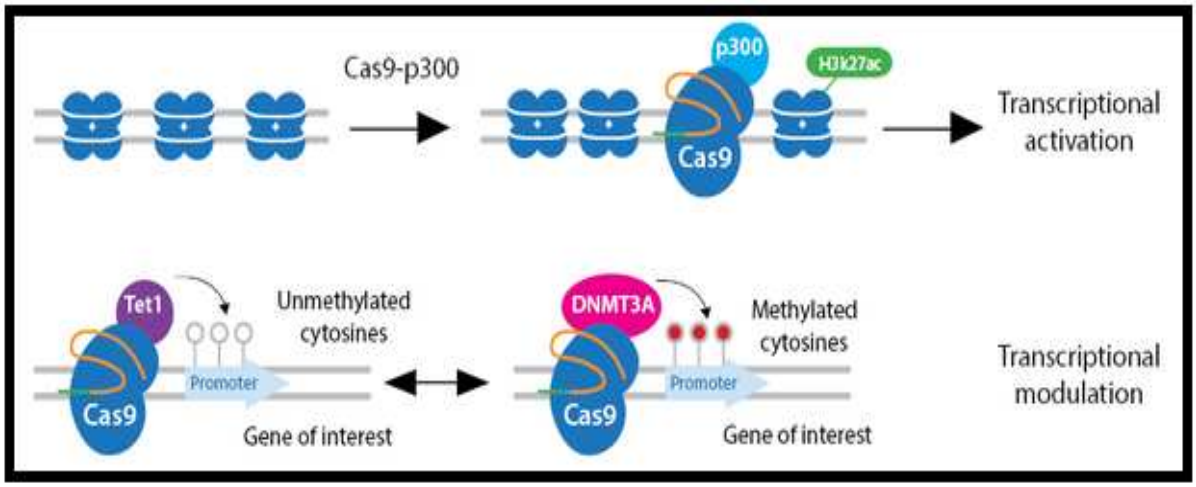

Fig. 8. Epigenetic Modifications with CRISPR (Guide 2018).

Selection of gene and genetic element to be modified

Target gene and its specific region to be manipulated must be identified. Selection of target region however, bases upon the what type of gene manipulation one desires. Each gene manipulation requires specific region of gene to as a target.

Target for Activate/Repress: promoter region of gene of interest.

Target for Knockouts: 5′ exons, exons closer to the N-terminus. Alternatively, exons coding pre-recognized essential protein domains.

Target for Edits: Point ≤ 10 base-pairs away from where the actual edit is desired.

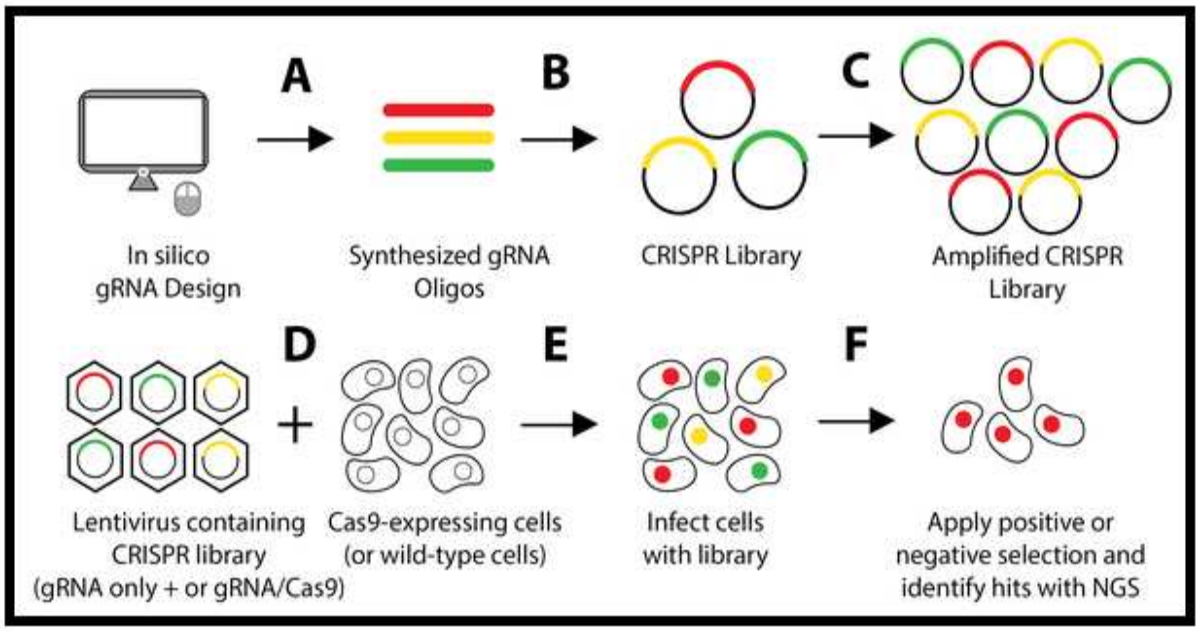

Fig. 9. Genome-Wide Screens and CRISPR Pooled Libraries (Guide 2018).

On-target and off-target anticipated-activity based selection of gRNAs

A perfect gRNA would be the one with: Complete homology with the target sequence; No homology with any locus in genome other than target locus. But, in reality either one of these two conditions or both of them do not fulfill. The sites in genome other than the target locus, with which gRNA possess homology are known as off-targets and the target locus being on-target. Researcher should consider all possible measures to reduce cleavage at off-targets (of- target activity) and simultaneously enhance cleavage at on target (on target-activity). Various gRNA designing programs provide facility of predicting the off-target and on-target gRNA activity and thereby, facilitating the gRNA design. Additionally, validated gRNAs contained with-in plasmids can be used to save both time and energy.

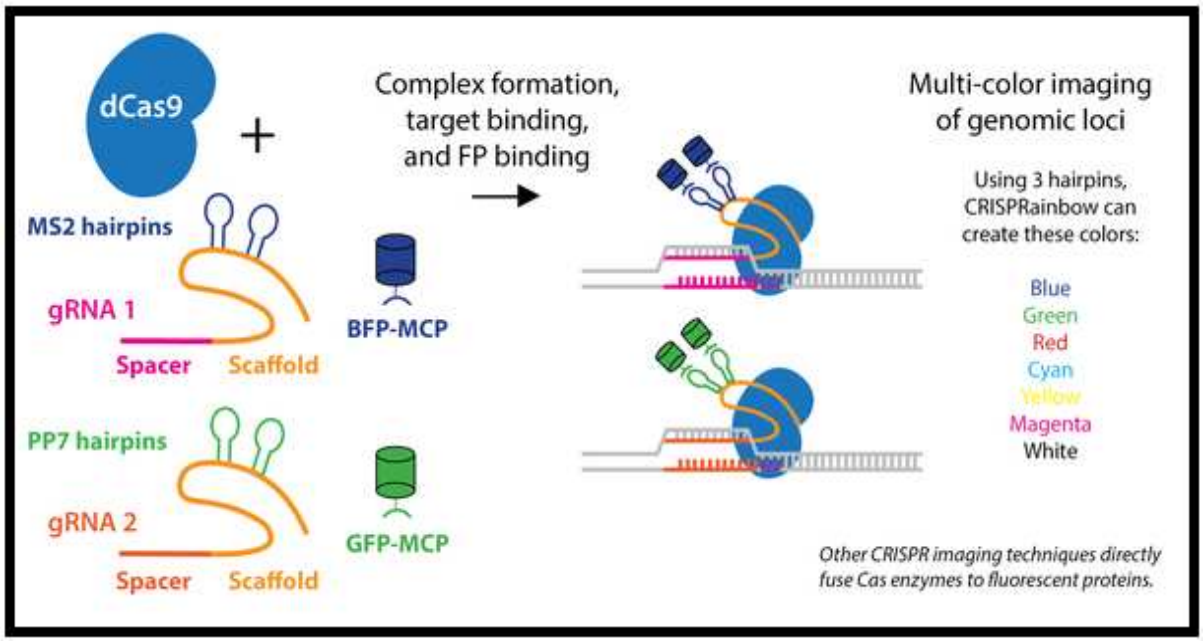

Fig. 10. Visualization of genomic loci using fluorophores (Guide 2018).

Synthesis and cloning of necessary gRNAs

When you have identified the target sequence, you navigate towards designing the desired gRNAs. Then comes the turn of cloning these gRNAs into specific vector of interest; Strategy of cloning depends upon the choice of vector.

Delivery of Cas9 and gRNA

In order to deliver the gRNA and Cas enzyme into the target cell, one has to select an expression system and delivery method, which requires to have high compatibility with the expression system. Success of manipulation is very often influenced by the cell type and delivery method. Components of expression system vary with each type of delivery method, which include: Mammalian expression vector-based transfer, Lentiviral transduction, AAV transduction, RNA delivery of Cas9 and gRNA and electroporation or transfection with Cas9-gRNA RNP (ribo nucleoprotein) complexes.

Validation of genetic modification

After the successful delivery of Cas and gRNA into the target cell, one has to validate whether the desired the genome manipulation has occurred or not; There is a variety of possible ways by which this verification can be done, choice would depend upon researcher's particular application. Some of the validation ways are:

NHEJ repaired DSBs: Mismatch Cleavage Assay.

HDR repaired DSBs: PCR-RFLP.

HDR or NHEJ repaired DSBs: PCR-Gel electrophoresis, PCR-Sub-cloning- Sanger sequencing, PCR-Next generation sequencing.

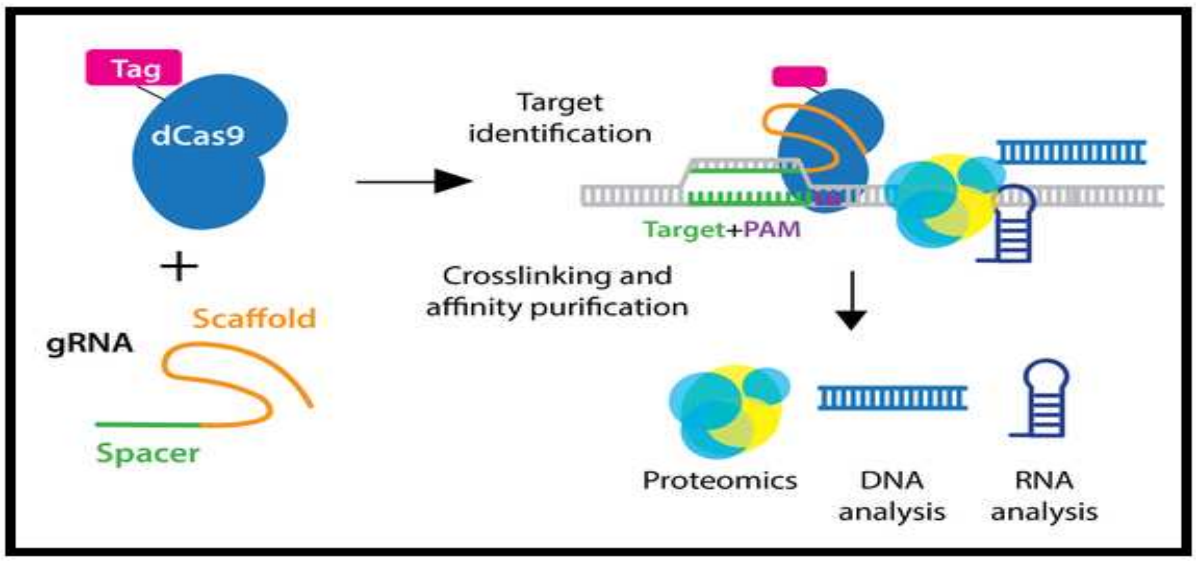

Fig. 11. Purification of genomic regions with dCas9 (Guide 2018).

Comparison ofCRISPR-Cas9 to other genome editing tools

There are four categories of genome editing techniques which are being used for manipulating genes in model organisms and humans such as for generating knock-out or elevation/repression of target gene expression for therapeutic purposes. These techniques are namely, ZFN (zinc finger nucleases), TALENs (transcription activator-like effector nucleases), engineered mega-nucleases and CRISPR system having the guide-RNA and Cas9 enzyme. ZFN technology involves targetable engineered cleavage proteins whereas, TALENs are proteins similar to ZFN and they are derived from *Xanthomonas* bacteria; Both of them work through the FokI. Meganucleases are on the other hand both, naturally occurring (in microorganisms) and engineered (having unique sequences) (Sander and Joung 2014; Cox *et al.* 2015; Maeder and Gersbach 2016).

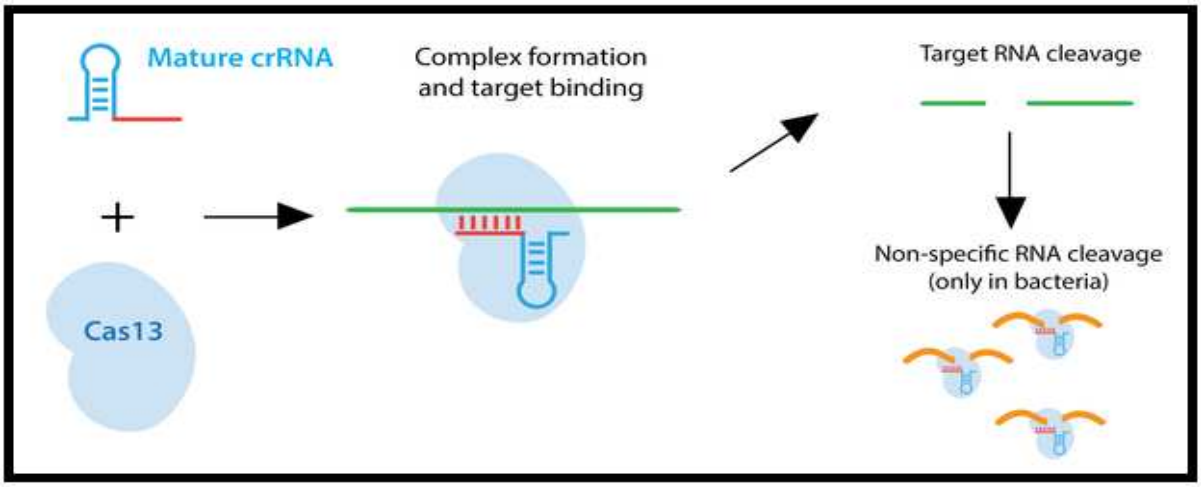

Fig. 12. RNA Targeting (Guide 2018).

Ethical concerns and policy hurdles

There is a healthy contrast of CRISPR/cas9 system policy regulations around the globe. Use of this technology to edit non-reproductive cells is less controversial than the use for germline modification. In UK researchers are allowed to genetically manipulate human embryos with the use of CRISPR/Cas9 and sister techniques but, there is no permission for the implantation of these modified embryos(Callaway 2016).In USA, it is allowed to use

genome editing techniques to modify foods and crops, for which there are well elaborated acts such as the Agriculture Risk Protection Act of 2000 (McHughen and Smyth 2008) and, due to high emergence of false technology, FDA has started making new policies(Brown 2017).Whereas, China has no religious and any other hurdles against using these genetic modifications to alter the human genotypes (Cyranoski 2017) and therefore, China has very few policy barriers against the use of CRISPR/Cas genome editing (Peng 2016).In 2015, an International Summit on Human Gene Editing occurred in Washington where, national scientific academies of US, UK and China debated about the ethics of CRISPR based germline modification and they generated associated ethical and legal guidelines. They decided to begin an international forum for addressing ethical concerns and produce harmony across the scientific world. But, recently in China embryo genome editing with CRISPR/Cas9 was abandoned at its initial stage because the experiment produced off-targets and was not fully successful. So, there is a need for calling on a temporary ban on CRISPR/Cas genome editing and having a wide-ranging discussion about all the ethical concerns and challenges by involving scientific community and stake holders. Map should be drawn to know what do to and, what not to in future with this technology, so that therapeutic advances involving somatic cell manipulation may not get hindered.

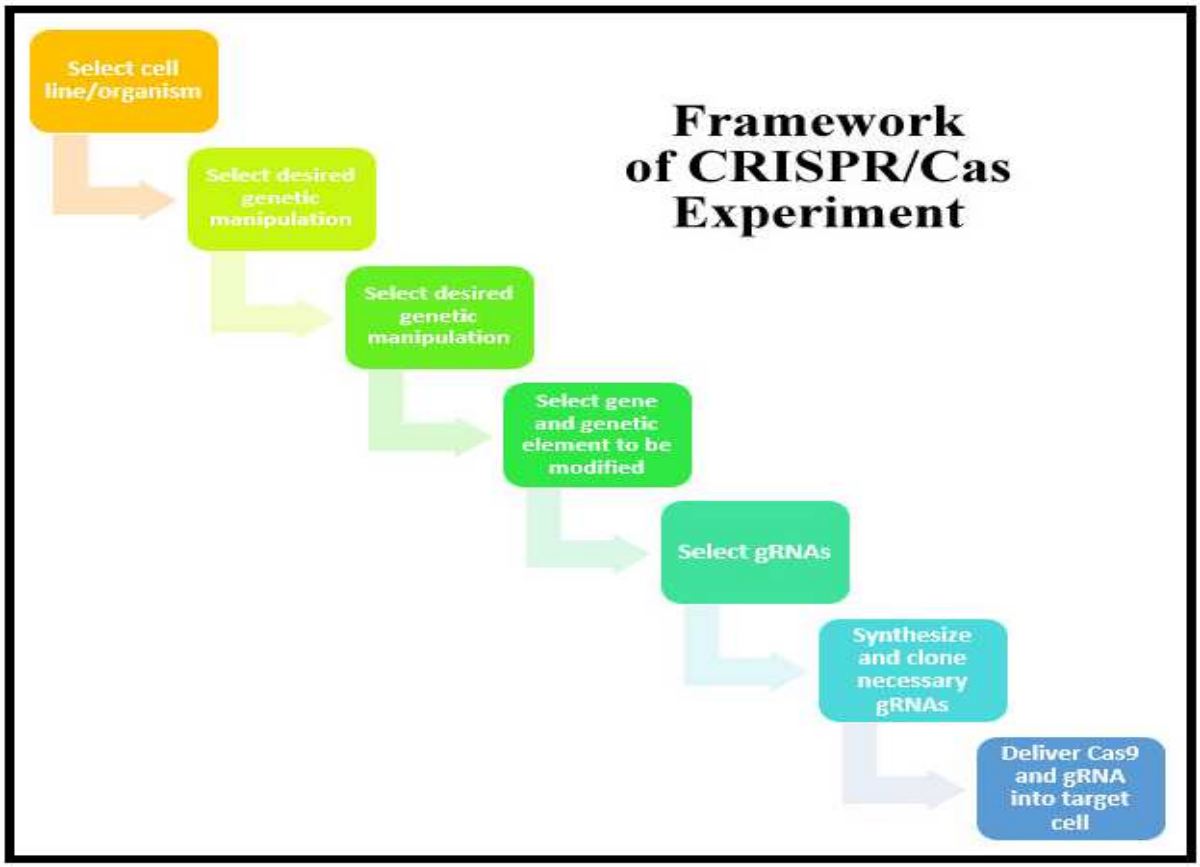

Fig. 13. General framework of CRISPR/Cas based genetic manipulation.

CRISPR-Cas9 based modification in humans

Somatic cell editing

In humans, CRISPR/Cas9 has been applied for the manipulation of genomes of somatic cells and, this has resulted in the favor of CRISPR/Cas9 as having a great potential of treating several human diseases which have some genetic etiological factor involved, e.g. cancer, hemophilia, hepatitis, etc.

Germline cell editing

Where most of the scientists in the word are interested in and debating about the potential use of

CRISPR/Cas9 system in editing human germline or reproductive cells, China has already used this tool to edit genes of 86 people. In 2015, Chinese scientists made first (Liang *et al.* 2015) and in 2016, second attempt of CRISPR based human embryonic modification (Regalado 2018). Whereas, US, and UK, have shared their plans for application of CRISPR/Cas9 on human embryos. But, it would still take scientists some years to make a routine use of CRISPR/Cas9 in humans.

Current Happenings about CRISPR

Identification of Pre-Existing Adaptive Immunity to Cas9 Proteins in Humans

Before directing CRISPR/Cas tool to clinal trials, researchers are have discovered that in humans there exist both adaptive cell mediated immunity and humoral immunity against Cas9 enzyme. This alarms for the necessity of using sources of Cas9 homologs other than Staphylococcus aureus and Streptococcus pyogenes bacterial species, as they cause serious infections in the humans, therefore there is high immune response against them.

Correction of DMD (Duchenne Muscular Dystrophy) mutations

DMD or Duchenne muscular dystrophy is an X-linked pediatric cardiac disease, which involves mutations in dystrophin gene causing progressive impairment, i.e. weakening and loss. DMD patients die usually before thirty because of cardiac and/or respiratory failure. Experiments with CRISPR/Cas9 have very intriguingly resulted in restoration of dystrophin gene expression and correction of crook mutations. Scientists have been successful in replacing abnormal gene copy with fully functional copy. In near future hopefully, CRISPR technology would help in personalized genetic treatment of DMD (Salmaninejad *et al.* 2018; Shimo *et al.* 2018).

CRISPR Gene Editing of Neurons in Prader-Willi Syndrome

Rare, unusual and lethal disease, Prader-Willi Syndrome (PWS) has been the new target of CRISPR/Cas interrogation. Genome imprinting of chromosome 15 results in PWS, which results in life threatening obesity and group of other problems. CRISPR/Cas9 has been proved promising in shutting down the *ZNF274* gene, producing ZNF274 protein which controls silencing machinery for imprinted section of maternal chromosome 15.

Clinical trial testing for CTX001, in Beta Thalassemia

CRISPR Therapeutics, pharmaceutical company has aimed to begin clinical trial for application CTX001, in β-thalassemia patients in Europe. CTX001 CRISPR based gene modification which involves genetic engineering of therapy β-thalassemia patients to produce increased levels of fetal hemoglobin in erythrocytes. Elevating fetal hemoglobin, will lessen down the transfusion requirements for β-thalassemia and it would be equally helpful for patients of sickle cell anemia.

Treatment therapy for Transthyretin Amyloidosis

Transthyretin amyloidosis is an autosomal dominant hereditary disease which is also known as Familial amyloid polyneuropathy. It involves mutation in the TTR (Transthyretin-related) gene which causes formation and deposition of protein aggregates, in body organs across the body resulting in numerous toxicities and eventually death from cardiac and renal toxicities. CRISPR/Cas9 based correction of desired mutation by using antisense oligonucleotides (ASOs) and siRNAs (Small interfering RNAs) is being done to change splicing and knocking down TTR gene expression (Beaudet and Meng 2016).

Injected treatment for Leber Congenital Amaurosis

Leber congenital amaurosis involves retinal dystrophy, especially the subtype LCA10. LCA10 occurs due to mutations in the *CEP290* gene. CRISPR/Cas9 mediated targeted precise genomic deletion has emerged as promising therapeutic method for the cure of LCA10, particularly due to splice mutation in *CEP290* gene. Researches were up-to finding solution for immunity against prolonged expression of enzyme SpCas9 and they have come up

with a CRISPR-Cas9 system which self-limits the time required for SpCas9 expression (Ruan *et al.* 2017).

Gene editing in Sickle Cell Disease

Two different versions of CRISPR editing, CRISPR/Cas9 and CRISPR Cpf1, are being used for the correcting the mutation in the ß hemoglobin (*HBB)* gene, which causes Sickle cell anemia. Cpf1 has found to be more useful than Cas9 as it recognizes and cuts more sites within the gene than Cas9.

These two nucleases have broadened the therapeutic horizon for hematologic disorders.

Gene editing in Inflammatory Bowel Disease

Scientists are working for the possibility of utilizing CRISPR/Cas gene editing tool in treatment of Inflammatory Bowel Disease (IBD). *C1orf106* gene is associated with IBD, actual role of this gene is still vague but it supports repairing damage to the lining of intestinal tract (epithelial cells) by controlling amount of the Cytohesin-1 (guanine nucleotide exchange factor).

A variant of *C1orf106* found to associated with IBD causes reduction in expression of Cytohesin-1 and thereby, reduction in ability of stabilizing damaged epithelial cell lining. The use of CRISPR/Cas9 system for upgrading the stability of this gene would a promising therapeutic technique to prevent and regain the integrity of the intestinal lining of IBD patients.

CRISPR Tomorrow

CRISPR based correction of various disease-causing mutations

Recently, CRISPR/ Cas technology has been largely used for studying and correcting mutations causing different diseases in humans; Diseases being studied where, mutation is the key etiological factor include cystic fibrosis, Barth syndrome, DMD, hemophilia and thalassemia. Mutations range from point errors used to large deletions. In future therapeutic power of CRISPR/Cas gene editing may open doors for correction of more disease-causing mutations (Cai *et al.* 2016).

CRISPR based elimination of disease causing microbes

CRISPR/Cas tool has successfully removed the DNA of HIV virus from the human (AIDS patient) genome (Kaminski *et al.* 2016). Efforts are also being made to eradicate other microbes such as those causing hepatitis and herpes.

Engineering new drugs

Pharmaceutical companies are trying to develop drugs based on CRISPR technology for the treatment of cardiac diseases, blindness, blood disorders and other rare inherited disorders. Bayer AG and CRISPR Therapeutics are among leading companies exploring the ways to create new drugs with CRISPR/Cas tool.

CRISPR based species resurrection

CRISPR technology could to miracles like revival of lost species. Scientists have already claimed to have developed an embryo of elephant-mammoth hybrid and they are trying to implant it into elephant and take it to full-term. CRISPR in this manner is being used to combine the genetic material of elephant and mammoth.

CRISPR aided creation of new healthier foods

Scientists are using CRISPR gene editing technology to modify foods and such foods are different from traditional GMOs, having no foreign DNA insert. CRISPR edited foods would be safe and have higher yields due to less more disease resistance and stress tolerance.

CRISPR based eradication of dangerous Pests

Favorably biasing the inheritance of a desired gene (gene derive) through CRISPR technology in mosquitos has generated deep apprehension about complete relief from malaria. The method involves targeted disruption of crucial genes required for female fertility in the malaria mosquito by CRISPR nuclease; Homing ensures inheritance of mutated genes in offspring generations (Hammond *et al.* 2017). Researchers are exploring ways to use gene

derives to control other diseases caused by mosquito particularly dengue and, diseases caused by other pests like ticks.

CRISPR based fixing of donor organ shortage

CRISPR/Cas tool has led to the reignition oftransplanting porcine tissues and organs into humans, as it has been done in past, replacement of islet cells in diabetes type I patients with porcine islet cells. To meet the shortfall of tissue/organ transplantation, CRISPR/Cas based xenotransplantation might prove to be less immunogenic and non-risky, less transmission of porcine retroviruses (Fung and Kerridge 2016).

CRISPR based alternative to petroleum

Many microbes such as bacteria, yeast and algae have an ability to produce PHA (polyhydroxy-alkanoates) which are bio-polyesters. PHA possess properties comparable to petroleum plastics for example polyethylene or polypropylene (PP). CRISPR/Cas based precision genetic modification and controlled gene expression in variety of model organisms has not only raised hopes for its PHA promise delivery but, also generated possibility of novel resources for various other biofuels. Genetic engineering in non-model organisms may contribute to further reduction in cost production (Li *et al.* 2017; Tao *et al.* 2017).

Development of designer pets and service animals

Yes, we are on our way to customize genome of our pets. Thanks to CRISPR, evolution is now under human control and we can make our pets smarter and even live longer. CRISPR aided germline editing has been done in pigs and dogs (Zhou *et al.* 2015).

Production of hardier livestock

CRISPR/Cas9 gene manipulation has allowed scientists to get rid specific genes in livestock which resist their hair and muscle growth thereby, enabling production of higher stocks of both meat and wool. In future CRISPR/Cas might lead to further expansion of livestock industries.

Conclusion

CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) has sprung up as a powerful tool for targeted gene editing and extensively captivating biomedical research world nowadays.

This adapted gene editing machinery, is being used to study and treat various human diseases which involve genes and immunity. Furthermore, its use in enhancing genetic code of crops and livestock is also gaining much glamor. Unlike somatic cells, the use of CRISPR/Cas9 based gene manipulation in germline cells is controversial. Although it is opening new doors to therapeutics and personalized medicine, the caution flags are also high. A way must have found to certify that the CRISPR/Cas9 would bind and cut accurately, such as: designing more specific gRNAs and employing Cas9 which would generate a nick rather than a DSB.

There is a need of a lot of work pivoting on controlling CRISPR/Cas9 'off-target' activity, so that all the precious parts of genome other than target sequence would remain saved from unwanted manipulations. After reviewing all aspects and consequences of CRISPR, we believe that Instead of getting caught up in the allure of CRISPR, scientific society needs to be more conscious and concerned about the ethical consequence of this prodigious scientific and technological development. Therefore, it might take more years to routinely use CRISPR/Cas9 in humans.

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