A New Era of Genome Modification

Liz Hampton

Honors Project with Dr. Pamela J. Langer,

Department of Molecular Biology

In partial fulfillment of the Honors degree, to be awarded

with a B.S. in Molecular Biology

University of Wyoming

Laramie, Wyoming

May 10, 2017
Table of Contents

Abstract

History of genome modification methodologies

   Molecular technologies related to CRISPR/Cas9

   Zinc finger nucleases

   TALENs

   CRISPR/Cas9 system

Potential applications of the CRISPR system

   Genome modification

   RNA editing

   Biomedicine

   Genome function studies

Impact of CRISPR technology on society

List of Figures and Tables

List of Works Cited
Abstract

Four decades ago, the advent of technology that could be applied to genetic engineering stimulated the hope that one day we would be able to ‘fix’ genetic disorders or inhibit cancer growth by replacing defective genes or introducing helpful genes into a person’s genome. That hope was followed by frustration with the limitations of the technology until the recent development of a new strategy used in the CRISPR/Cas9 system, which is considered one of the most important developments in the field of genome modification to date. CRISPR technology can be used both for genome modification and post-transcriptional editing through RNA editing. Envisioned applications of the CRISPR system range from basic research on cancer and the study of essential genes to treatment of genetic diseases and cancer. However rather than a panacea for the treatment of intractable medical problems, the technology has presented potential negative consequences. Scientists have called a moratorium on the use of CRISPR technology until it was better understood. Furthermore, policy makers have preemptively banned the use of CRISPR in human systems. In spite of these fears, China has moved forward on studying CRISPR in human embryos anyway, though esteemed journals such as Nature, Science, and others, will not publish their studies due to ethical concerns. This literature review will describe the mechanics of the CRISPR technology in DNA modification and RNA editing, some of the current research applications and challenges, and finally, potential human applications and the need for regulatory oversight.
History of genome modification technologies

With the elucidation of the three-dimensional structure of DNA by Watson and Crick came a sense that the keys to life itself had been discovered. This sense fueled the hope that we as a species could do anything from clone other organisms to treat genetic diseases and cure cancer. Scientists have since used knowledge gained from studies of DNA to engineer adenoviruses to contain a correct version of a mutated gene, instead of the original viral genome. These experiments operated on the hypothesis that by injecting patients with the modified adenovirus, instead of getting sick, the correct version of a mutated gene causing their disorder would be inserted into the genome, and the problem would be solved. This method was crude and often triggered an immune response, resulting in a poor success rate. This is to say nothing of the complexity of editing a eukaryotic genome and considering all the epigenetic factors, many of which are still not well understood. The very complexity of eukaryotic genome editing has limited the applications of the technology to very localized environments such as the eye, where it is quite successful in areas such as treating blindness in dogs (Zou 2015).

Relatives

There are many related technologies that are both contemporary with and precede CRISPR/Cas9, including nucleases and meganucleases. These technologies also rely on the ability of the enzyme to cut DNA and the cell’s immediate survival response of repairing the cut. Ideally, this will lead to the insertion of a gene (a corrected version of a mutation to cure a genetic disease, or a gene of interest for research purposes), but can be exploited to create natural mutations (as CRISPR can). The latter is also incredibly useful for research purposes, as it helps provide clues about the utility of the gene under study (Wall, D. 2017). These systems are
derived from eukaryotic systems and are thus better suited to use in eukaryotic systems as opposed to the prokaryotic CRISPR/Cas9 system, though they are somewhat less effective than CRISPR, and several studies have been focused on improving their efficacy and uptake by cells and reducing their tendency to make off-target genomic changes (changes that are completely unintended, somewhere in the genome other than the original target). Given the size of eukaryotic genomes compared to prokaryotic genomes it is more common for genome editing devices such as meganucleases, nucleases, etc., to find an acceptable site for enzymatic activity that was not originally intended (Ellis et al. 2013; C. He et al. 2014).

**Meganucleases**

Technologies used to modify the genome include not only the meganucleases, but also the zinc-finger nucleases and the transcription activator-like effector nucleases, or TALENs. The meganucleases are known for their very large recognition sites, such that the approximately eighteen base pair site can only occur by chance in a genome twenty times the size of what humans possess. This recognition site can be modified to choose specific sites in the genome, for research and genome modification purposes (Grizot et al. 2009; the term “genome modification” first introduced in Resnik, Langer 2001). It has been found that meganucleases are toxic at high levels, but can be introduced at low levels and have desirable effects on the cells under study (C. He et al. 2014). In conjunction with viruses, meganucleases have been shown to allow normal viral growth in tumors and inhibit their growth. Generally meganucleases respond to factor p53, which allows for self-disruption of viral DNA and effective immunity, but in some tumor cells the factor is not expressed, so the virus grows normally and lyses the tumor cells (Gürlevik et al. 2013).
Zinc-Finger Nucleases

The zinc-finger nucleases possess a motif that allows it to interact with the DNA, wrapping around it and following the major groove until finding its restriction site for DNA cleavage. This type is artificial, created by fusing a zinc finger binding domain and a nuclease in order to improve interactions with the DNA, especially in complex genomes (Maeder 2008). Zinc-finger nucleases were studied in melanoma tumors, where they were used to target genes involved in the apoptosis process. To do this, they targeted the genomes of infiltrating lymphocytes in order to increase the number of surface proteins involved in apoptosis, increasing the signal load to the cells in order to override the mechanisms standing in the way of these proteins’ normal functioning (Beane et al. 2015). Zinc-finger nucleases have also been used in HIV research, where they knocked out specific receptors on white blood cells that effectively make them immune to the virus (Digidu et al. 2014). In tobacco, zinc-finger nucleases can be used to create a double-cut in order to insert a cassette of genes into the genome as a proof of concept study selecting for kanamycin resistance and using a visual marker (red fluorescent protein) to demonstrate that transformation has occurred. This may one day be used to improve photosynthesis, crop yields, insect resistance, and other similar problems affecting crops (Schneider et al. 2015).

TALENs

The TALENs mimic transcription activators, and are in fact fusions of TAL effector binding domains and DNA cleavage proteins. The effector segment of the protein can be made to bind to a desired sequence within the genome, allowing the exonuclease segment to make cuts in
the DNA for knockouts or genome modification (Boch 2009). They are native to eukaryotic systems and are therefore less likely to be recognized as a foreign protein than Cas9 by the immune system (Osborn 2013), leading to reduced side effects in eukaryotic cells. One such side effect was that Cas9 often led to off-target mutagenesis due to the larger genomes, where it was more likely that the site programmed into the crRNA would appear by chance. Like Cas9, TALENs can be programmed with deoxyribonucleotide primers to direct them to the site of desired repair, mutagenesis for study, and so forth, and for up to about 7.8 kilobases, mutagenesis and deletions were very efficiently achieved (Wang 2014). In one case, by deleting specific genes researchers were able to create a specific line of human tumor cells that allow them to study cell differentiation and tumor formation (Udhe-Stone 2014).

**CRISPR/Cas9**

The CRISPR/Cas9 system was discovered independently in three different parts of the world, and it was soon clear that the system was an adaptive immunity system for bacteria. It has also been shown that higher numbers of spacers that have been shown to match various phage that have infected the bacterial cell in the past. The number of spacers is correlated with a greater immunity for the cell, so with each new infection, a new spacer is created. These spacer sequences flank repeated sequences that, as RNAs, guide Cas proteins to the foreign DNA for digestion by the enzyme. The infection is essentially stopped before it can fully begin. The repeated sequences were first discovered in 1987 when part of the sequence was accidentally cloned to the *iap* gene, which governs alkaline phosphatase isozyme conversion (Ishino et al. 1987). In 1993 researchers found the repeats in *Mycobacterium tuberculosis*, with great diversity between strains. This allowed them to develop a typing method called Spoligotyping, a rapid,
PCR-based method which is still used today to identify and analyze the repeats in *M. tuberculosis* and other species (van Soolingen D, de Haas PE, Hermans PW et al. 1993). Simultaneously, similar repeats were observed in archaea (specifically *Haloferax* and *Haloarcula*) by Francisco Mojica, who studied their function and determined that they played a role in the segregation of DNA during cell division. This conclusion was later found to be incorrect, but he based it on the fact that in *Haloferax volcanii*, plasmids containing identical repeats could not coexist (Mojica 1993). Later studies have tried to manipulate the CRISPR system in order to determine if a phage can develop immunity to the cell’s natural defense system (Mojica et al. 2005), as well as elucidating the process behind inserting new spacers and repeat sequences (Pourcel et al. 2005).

**Mechanism**

*Native*
**Fig. 1: The CRISPR mechanism as it operates in bacterial cells.** There are two cellular responses to viral DNA. For novel infection, Cas proteins are produced to degrade the viral chromosome to insert a spacer into the array. For recurring infections, a different Cas protein is guided by the spacer, and the enzyme degrades the viral genome (Horvath, Barrangou 2010).

**Spacer Acquisition**

The native CRISPR system is triggered by the insertion of viral DNA into the cell’s cytosol. Viral DNA tends to be linear, whereas the bacterial chromosome is circular, and this makes it easy for the cell to identify the viral DNA as foreign, or “non-self” (Wall, D. 2017). This signals to the cell to make RecBCD complex proteins that bind to the foreign DNA and degrade it. The proteins Cas1 and Cas2 take one of these segments of DNA and perform a staggered cut in the CRISPR array at the terminal repeat sequence (Swarts et al. 2012). This staggered cut allows for the insertion of the new spacer DNA, and currently unknown
polymerases and ligases repair the damage, forming a new repeat sequence in the process (Wall, D. 2017). The spacer includes the protospacer adjacent motif (PAM) sequence, which serves a number of functions in the CRISPR system and bacterial immunity at large.

**Protospacer Adjacent Motifs**

Interestingly, which sequence is chosen to become the new spacer is not random. Instead, they were adjacent to what are known as protospacer adjacent motifs (PAMs). These sequences determine where CRISPR is active, but they also play a role in distinguishing self-DNA from non-self DNA in Type II CRISPR systems. If a section of DNA can base pair beyond the PAM sequence, that section of DNA is self, and this signals that degradation cannot proceed. However, if base pairing beyond the PAM sequence does not occur, this indicates the DNA is non-self and should be degraded (Bolotin et al. 2005). Furthermore, in certain systems, the PAM sequence plays a key role in spacer acquisition (Swarts et al. 2012), implying that the machinery creates overhanging repeats after the second-to-last repeat and within the PAM sequence itself. This does not, however, hold for all systems and microbes (Shah et al. 2013).

**Biogenesis**

When the cell is infected again, or by a phage for which a spacer already exists in the genome, the cas genes are made into proteins, and the CRISPR array is made into a long pre-crRNA which contains all the spacers and repeats in the array (Marraffini, Sotheimer 2010). Cas proteins cleave the pre-crRNA into mature crRNAs, and while this occurs on all systems, the precise mechanisms vary from system to system (Gesner et al. 2011). Different systems cleave the pre-crRNA in different ways, from stem-loops (Haurwitz et al. 2010) to cleavage taking
place just upstream of the repeat sequence through the RNA wrapping around the enzyme (Wang et al. 2011). Some systems use a different protein entirely for RNA cleavage, RNaseIII in type II systems, which use small RNAs complimentary to the repeats, known as trans-activating crRNAs or tracrRNA (Dugar et al. 2013). The tracrRNA and the crRNA base pair and form a double-stranded RNA sequence, forming a perfect target for RNaseIII for cleavage, resulting in crRNAs, with a slightly truncated spacer (Gasiunas G et al. 2012).

Interference

The individual crRNAs bind to other Cas proteins and guide them along the strand of viral DNA. Here again the PAM sequence becomes important, as it is recognized on the complimentary strand to the crRNA, meeting one of the two requirements for the system, the other being crRNA annealing. In Type I systems, annealing results in a conformational change, recruiting an additional protein, Cas3, for DNA degradation, whereas Type II systems rely on Cas9 (Gasiunas G et al. 2012). Cas9 requires both tracrRNA and crRNA for proper functioning, and its cleavage mechanism requires that the PAM sequence base pair properly with the phage genome (Zhang et al. 2012; Hale et al. 2009). Because the PAM sequence is recognized on the same strand as the spacer DNA used by Cas proteins for guidance, the fact that self DNA can base pair with the guide RNA of the Cas9 system beyond that point is very important. This mechanism allows the cell to protect itself from the degradation of its own genome (what amounts to suicide) (Wall, D. 2017).
Engineered CRISPR systems rely solely on the multifunctional protein Cas9, which is easier to maintain in varied systems than large complexes of many proteins. Furthermore, a designed CRISPR system has only one crRNA, the repeat sequence needed to interact with the Cas9 protein, and an engineered “spacer” sequence targeted to a gene of interest. This even works in eukaryotic systems, because the Cas9 system does not discriminate between alleles of a gene. When the DNA is cleaved, there are three possible outcomes, which under different circumstances may all be desirable. The cell patches the DNA together with a small indel mutation that renders the gene inactive or otherwise defective. This is useful in research fields when trying to determine the gene’s function; what is a cell’s phenotype when that gene is inactive? A second outcome is created when the Cas9 and crRNA are inserted with a piece of DNA, and when the cell needs to repair its genome it initiates homologous recombination with that piece of DNA, inserting it. This is useful in medicine, for correcting a variety of genetic diseases and for studying the genomes of cancers (Wall, D. 2017). However, because of the immune system, this method hasn’t gone very far in most areas of the body, but studies using this method in the eyes of dogs to treat blindness have been very successful (Zou et al. 2015). A third outcome is the result of two different Cas9 systems, each with their own guide RNA. The result of this is considerably larger indel mutations than with one single Cas9 protein, which is also useful in research contexts looking at gene function, and in fact could be used as a workaround to leaky expression and similar problems caused by other point mutations. If the entire gene is deleted from the genome of a cell, then there is nothing to express. True null mutations serve as negative controls in many settings (Wall, D. 2017).


Applications

**Genome Modification**

The discovery of CRISPR and its further study opened the door to use it for potentially editing genes deliberately. A Type II system is used for this due to the fact that it is comprised of the RNAs and a single multifunctional protein rather than a large complex, though the system is incredibly stripped-down version compared to the natural version of the system. Each element in the system has a specific function. The crRNA contains not only the guide RNA to the correct segment of the genome, but also a region that forms a hairpin loop structure allowing it to interact with the tracrRNA in order to form an active complex. Sometimes these are formed into one RNA, called the single-guide RNA or sgRNA. The RNA structure interacts with the protein Cas9, which is able to interact with DNA in a variety of ways. There are versions that cause single-stranded nicks, double strand breaks, or merely bind to the DNA, among other possible functions. The repair template is optional, but in a system for the modification of DNA it seems obvious. This is the DNA that guides the repair process toward the aim of its own insertion.

The CRISPR/Cas9 system is typically inserted into cells as a plasmid (Ran et al. 2013), and needs to be redesigned for each use. A single guide RNA is in fact good for only one spot in the genome, and unless multiple studies or experiments are being done on that very spot, the guide RNA cannot be reused. Furthermore, the repair template must also be designed each time in order to overlap in that precise spot for the cell’s repair machinery. Furthermore, it has been found that the efficacy of the system in eukaryotic cells is between approximately thirty and sixty percent. Despite these, however, the system is highly faithful and remarkably simple, making it a breakthrough technology in its field.
Furthermore, several methods currently exist where Cas9 is modified to edit the genome in response to an external trigger (the easiest such thing being light, the second easiest being various small molecules) (Nuñez, Harrington, Doudna 2016; Nihongaki et al. 2015). The methods for this include photoactivatable CRISPR systems comprised of light-responsive protein partners fused to a dCas9 in order to activate the gene (Polstein, Gersbach 2015), fusing related light-responsive domains to constructs of split-Cas9 (Wright et al. 2015), the use of unusual amino acids as markers (Hemphill et al. 2015), or the use of photocleavable elements incorporated into the guide RNAs (Jain et al. 2016). The various small molecules that have been used to similar effect include the use of 4-hydroxytamoxifen (4-HT) (Oakes et al. 2016), intein-linked Cas9s that respond to 4-HT (Davis et al. 2015), or Cas9 that is only 4-HT responsive when fused to ERT2 domains (Liu et al. 2016), among other categories of small molecules. They can also be used to improve homology directed repair (HDR) (Yu et al. 2015) mostly by inhibiting the non-homologous end-joining (NHEJ) pathway (Maruyama et al. 2015).

**RNA Editing**

Genome modification is the most well known of CRISPR applications, and what everyone tends to think of when they think of the CRISPR/Cas9 system. However, any point along transcription and translation can be tampered with to cause mutant phenotypes. For instance, in 2016 it was discovered that a CRISPR system from a mouth bacterium could edit RNA, and they showed that when they edited other CRISPR systems with these differences, the resulting system, C2c2, could defend against RNA viruses. There is a huge focus on using this new system against viruses such as HIV and poliovirus, and very much like the CRISPR/Cas9 system that edits DNA, C2c2 can be edited to edit any RNA molecule (Zimmer, 2016, *The New*
York Times). This could open the door for CRISPR’s use to expand to RNA viruses as well, single- or double-stranded.

**Biomedicine**

CRISPR’s great editing capabilities open up numerous new avenues for anti-viral medicine. What are termed “RNA-guided nucleases” could target everything from antibiotic resistance to virulence proteins and capsid synthesis, and beyond. This represents antimicrobial therapy and new selective pressure on bacterial populations (Gomaa et al. 2014; Citorik, Mimee, Lu 2014), as well uses in humans; affected genes have ties to diseases of muscle differentiation, inflammation, fetal hemoglobin, as well as cancer (Pennisi 2013). Furthermore, it has been shown that CRISPR is effective against Epstein-Barr Virus (EBV), showing promise at limiting its replication, removing it from cancer cells, cleaning out donor organs for immunocompromised patients, and blocking HSV-1 reactivation, preventing eye infections and cold sores (van Diemen et al. 2016). Besides cleaning up human organs for transplant, CRISPR is showing signs of being able to reopen the door of using animal organs for transplant by cleaning out retroviral DNA from the animal’s genome (Yang 2015), which in the future will greatly alleviate the waiting times for people who need those organs. Another interesting thing CRISPR can remove from transplants is MHC class II proteins found in blood vessels which often cause transplant rejection (Abrahimi et al. 2015). The CRISPR system is already showing great promise in the realm of medicine and could greatly alleviate the stress on the organ donation infrastructure to help reduce waiting times for people who greatly need organs, and the risk of rejection for people who already have organs.
Genome Function Studies

As stated, CRISPR is incredibly useful to studying gene function. A series of studies in 2015 sought to identify how many human genes were essential to our biology. The results showed that between 1,600 and 1,800 such genes were essential, out of approximately 20,000; because of their status as required, they are less likely to carry deleterious mutations and more strongly active, also they are more likely to have counterparts in other species that are just as essential to their biology. Interestingly, these studies found genes that are essential to four cancer-cell lines that healthy cells consider expendable, which drugs can be made to target without causing lasting harm to the rest of the body (Yong, 2015, The Atlantic). The functions of slightly under twenty percent of these essential genes are as yet unidentified. A study that attempted to determine which genes made groups of cells resistant to a melanoma drug operated on the method that each instance of genome manipulation was a “drug”, which opened up the entire genome to CRISPR regulation (Shalem et al. 2014). Essentially what this means is, each edit made by the CRISPR system is a potential supplementary drug or treatment to the melanoma drug under study which may enhance the latter drug’s effects on the cells. More broadly, however, this can apply to any disease, indicating that the CRISPR system used to treat, say, a heritable disease is, also, a drug for that disease. This has, interestingly, opened up the door to speculation that CRISPR can be used in a performance enhancing way in sports. While we cannot detect the edits themselves, or the Cas9 protein, sports regulators are investing in research into the metabolites produced by these edits to determine whether CRISPR/Cas9 has been used for performance enhancing purposes (Fischetto, Bermon 2013).
In Society

Moratorium

CRISPR has many more applications than discussed here, and is very much under study to this day. However the technology, and the concept of genome manipulation in general, elicits very strong feelings in people. Because CRISPR is so much more effective than other means of genome modification, to some it seems to represent a real danger to what it means to be human (already an incredibly tenuous definition, as some states have castle domain laws that consider one’s home something of an extension of the self; however our current society has a very absolutist definition about what it means to be human, seeing the human body as an absolute thing, with a definitive boundary. Anything that falls out of this is considered “other” and can be quite terrifying, representing a threat to general human identity). The idea that the genetics of humanity can be changed so easily causes a visceral reaction usually expressed as accusing the scientists of “playing God” whenever the subject is mentioned. Because of this, scientists, including a CRISPR co-inventor, have called for a worldwide moratorium on applying the technology, stating that “scientists should avoid even attempting, in lax jurisdictions, germline genome modification for clinical application in humans” until everything has been “discussed among scientific and governmental organizations” (Baltimore et al. 2015; Lanphier et al. 2015). The recommended approach, then, is basic research on the CRISPR/Cas9 system until it is developed enough for clinical work in humans (Wade 2015, The New York Times).

International Summit

An International Summit on Human Gene Editing was held in Washington in December of 2015, where members of the national scientific academies of Britain, the United States, and China met to discuss the ethics of editing the human genome, particularly in the germline. A
distinction between somatic cells, which limit the effects to a single individual, and germline cells, which possess the ability to alter that person’s entire line of descendants, was made. Furthermore, editing the germline of an individual was deemed irresponsible, due to the potential long-term effects both biologically (in terms of natural evolution of humankind, as well as interactions between genes and environment, which we still do not fully understand), as well as socially (such as considering the phenomenon known as Social Darwinism, which used to posit that people were wealthy, poor, white, black, or of some other group because they “deserved it” on a primarily undefined basis; it may come to mean something entirely different with these advancements in human genome modification technology) (National Academies of Sciences, Engineering, and Medicine, 2015).

**China**

Despite these various actions, Chinese researchers have decided to move forward in investigating CRISPR in human biological systems, specifically in nonviable human embryos. The study was an attempt to use the CRISPR/Cas9 system to correct a mutation that causes the lethal heritable disorder beta thalassemia (Liang et al. 2015). Both *Nature* and *Science* rejected the study due to its violations of their ethical requirements, despite that the researchers have stated that the technology is not ready for clinical use in reproductive systems (Nature 2015). A year later they made a second attempt to alter the human genome, targeting the CCR5 gene in an effort to make the embryo HIV resistant (Regalado 2016, *MIT Technology Review*).

**Policy**

Policy restrictions on this research vary globally. For instance, in the UK scientists were recently given permission to modify human embryos with the CRISPR/Cas9 system, with the stipulation that the embryos were never implanted, and destroyed after seven days (Callaway
The United States uses an elaborate interdepartmental regulatory system to evaluate genetically modified foods for public health and safety, through the Agriculture Risk Protection act. This act regulates, among numerous other things, genetically modified crops that use the DNA of any identified ‘plant pest’ or anything not categorized prior (McHughen, Smyth 2006).

In recent years, Yang has successfully used the CRISPR/Cas9 system to deactivate sixteen genes in a white button mushroom, and as he did not use any foreign DNA, the mushroom successfully passed US regulations (Waltz 2016, Nature News).

The USDA has sponsored a committee to consider future policy regarding genetic modification techniques, taking in commentary from the National Academies of Sciences, Engineering, and Medicine as well as special interest groups. The meeting was held on April 15 and considered possible advancements in genetic modification within the next five years, as well as what regulations such advancements would require (Ledford 2016, Nature News; Brown 2017, Gizmodo).

**Recognition**

CRISPR is a two-time runner up (2012 and 2013) for *Science Magazine*’s Breakthrough of the Year award, and won it in 2015. In 2014 and 2016, CRISPR was named an *MIT Technology Review* breakthrough technology.
Figures

Figure 1 – The CRISPR mechanism as it operates in bacterial cells. Pg 8.
Works Cited


Bolotin A, Quinquis B, Sorokin A, Ehrlich SD (August 2005). “Clustered regularly interspaced short palindrome repeats (CRISPRs) have spacers of extra chromosomal origin”.

Microbiology. 151 (Pt 8): 2551-61. doi: 10.1099/mic.0.28048-0. PMID 16079334.


Digidu, Chuka A. et al. “Simultaneous zinc-finger nuclease editing of the HIV coreceptors ccr5 and cxcr4 protects CD4+ T cells from HIV-1 infection”.


Cas9 system for rapid control of genome editing”. Nature Chemical Biology. 12 (11): 980-987. doi: 10.1038/nchembio.2179. PMID 27618190.


Qingjian Zou, Xiaomin Wang, Yunzhong Liu, Zhen Ouyang, Haibin Long, Shu Wei, Jige Xin, Bentian Zhao, Sisi Lai, Jun Shen, Qingchun Ni, Huaqiang Yang, Huilin Zhong, Li Li, Minhua Hu, Quanjun Zhang, Zhidong Zhou, Jiaxin He, Quanmei Yan, Nana Fan, Yu Zhao, Zhaoming Liu, Lin Guo, Jiao Huang, Guanguan Zhang, Jun Ying, Liangxue Lai,


Udhe-Stone, Claudia et al. “A TALEN-based strategy for efficient bi-allelic miRNA ablation in human cells”. RNA 2014. 20: 948-955. doi:10.1261/rna.042010.113


Yang, Luhan; Guell, Marc; Niu, Dong; George, Haydy; Lesha, Emal; Grishin, Dennis; Aach, John; Shrock, Ellen; Xu, Weihong; Poci, Jiirgen; Cortazio, Rebeca; Wilkinson, Robert A.; Fishman, Jay A.; Church, George (27 November 2015) “GENOME EDITING:Genome-wide inactivation of porcine endogenous retroviruses (PERVs)”. Science. 350(6264):1101-1104. doi: 10.1126/science.aadll91.


