In 2013, George Church and his colleagues at Harvard University in Cambridge, Massachusetts published "RNA-Guided Human Genome Engineering via Cas 9," in which they detailed their use of RNA-guided Cas 9 to genetically modify genes in human cells. Researchers use RNA-guided Cas 9 technology to modify the genetic information of organisms, DNA, by targeting specific sequences of DNA and subsequently replacing those targeted sequences with different DNA sequences. Church and his team used RNA-guided Cas 9 technology to edit the genetic information in human cells. Church and his colleagues also created a database that identified 190,000 unique guide RNAs for targeting almost half of the human genome that codes for proteins. In "RNA-Guided Human Genome Engineering via Cas 9," the authors demonstrated that RNA-guided Cas 9 was a robust and simple tool for genetic engineering, which has enabled scientists to more easily manipulate genomes for the study of biological processes and genetic diseases.

At the time of the publication of their article, the authors worked in Massachusetts. Church was a professor of genetics at Harvard Medical School in Boston, Massachusetts. He researched synthetic biology, or bioengineering, and made contributions to genomic sequencing techniques, techniques that enable scientists to read an organism's entire genome. The other researchers, except James DiCarlo, were PhD candidates at either the Harvard Medical School or Harvard University in Cambridge, Massachusetts. DiCarlo was a PhD candidate at Boston University in Boston, Massachusetts.

In their article, hereafter "RNA-Guided Human Genome," Church and his team discuss and use RNA-guided Cas 9 technology to modify the DNA of human cells. DNA is a double-stranded nucleic acid, which contains genetic information for cells. By modifying DNA, researchers can modify what the cell does and the proteins it produces. One way to do that is with RNA-guided Cas 9 technology, which uses RNA sequences to guide proteins to specific sequences of DNA. RNA is a single-stranded nucleic acid that translates genetic information from DNA into directions for making proteins. Researchers using RNA-guided Cas 9 technology select RNA sequences that match the DNA sequences they want to modify. They then use those RNA sequences to direct a Cas 9 protein to change the DNA. Cas 9 is a protein that cuts double-stranded DNA, leaving a space for a new sequence of DNA to be inserted. Church and his team used RNA-guided Cas 9 technology to modify specific sequences of DNA in human cells. The team describe that process and their results in "RNA-Guided Human Genome Engineering via Cas 9."

In their article, Church and his team first discuss the use of Cas 9 protein for gene editing,
which Jennifer Doudna and Emmanuelle Charpentier first proposed in 2012. The authors briefly delineate the mechanism of natural Cas 9 found in bacteria and the mechanism of the Cas 9 they used in their study. Next, the authors describe their methods for engineering Cas 9 to work in human cells and for designing a guide RNA. Church and his colleagues then report on their experiments testing RNA-guided Cas 9 using two different repair mechanisms called nonhomologous end joining repair and homologous recombination. Next, Church and his colleagues demonstrate their success for editing genes [3] that are introduced into human cells and genes [3] that are native to human cells. They also show differences in using RNA-guided Cas 9 instead of traditional, more complicated methods of gene editing such as TALENs. They state that the differences suggest that RNA-guided Cas 9 gene editing is a better method for gene editing than the traditional methods.

Church and his colleagues start their article with a description of their RNA-guided Cas 9 system as an engineered version of the natural Cas 9 system found in bacteria. As the authors note, the RNA-guided Cas 9 technology was developed from a natural bacterial system. Bacteria use a system called CRISPR to incorporate the DNA of viruses into their own genomes, which protects the bacteria from infection by the same virus again. In their article, Church and his team differentiate their technology from the system that bacteria use. The researchers note that normally, in bacteria, two types of RNA, crRNA, or CRISPR RNA, and tracrRNA, or trans-activating RNA, guide Cas 9 to a specified segment of the virus's DNA to cut, thereby inactivating the virus. The authors describe their engineered guide RNA as a fusion of crRNA and tracrRNA. The guide RNA has the function of directing Cas 9 protein to a target sequence. Once the Cas 9 is bound to the correct sequence indicated by the RNA, the Cas 9 protein cuts the double-stranded DNA. Then, new DNA with a modified sequence replaces the original cut-out sequence. Church and his colleagues describe that system as programmable because scientists can design RNA molecules to guide Cas 9 to cut any region of any organism's DNA, including the human genome [4].

After introducing their technology, Church and his colleagues discuss how they engineered the Cas 9 protein and RNA components of the RNA-guided Cas 9 system for use in human cells. Cas 9 is a protein found in bacteria that cuts DNA. Because Cas 9 is originally found in bacteria, George and his colleagues note some important modifications they made to enable Cas 9 to function in human cells. Unlike bacteria cells, human cells have nuclei, or central organelles that hold most of the cell's DNA. Therefore, in human cells, proteins must have a signal sequence that allows them into the nucleus [6]. Church and his colleagues explain that they added a signal to their Cas 9 protein so it could enter human cell nuclei. They note that the signal was derived from a human-infesting virus. By adding the signal to the Cas 9 protein, Church and his team enabled the Cas 9 protein to enter the nucleus [6] of a human cell and therefore to modify the DNA contained in the nucleus [6]. Next, Church and his colleagues describe their design of a guide RNA sequence to target a segment of the human genome [4]. The guide RNA enables the Cas 9 system to find the correct DNA segment and to edit the DNA.

Church and his colleagues go on to explain how they determined which repair method to use. RNA-guided Cas 9 technology takes advantage of cell repair mechanisms. When the Cas 9 protein cuts out a sequence of DNA, researchers rely on cell repair mechanisms to incorporate a new sequence into the genome [4]. The authors write that there are two methods cells use to repair DNA. The first method is homologous recombination. Homologous recombination requires a donor sequence provided by the scientist that matches the guide RNA and contains the desired sequence for gene editing. The guide RNA targets the site and
Cas 9 cuts it. The donor sequence that matches the guide RNA will then insert itself into the site to repair the DNA. The second is nonhomologous end joining. Nonhomologous end joining does not require a donor sequence. When DNA is cut by Cas 9, the DNA is left with two hanging ends. In nonhomologous end joining, those hanging ends join any available DNA in the cell that matches the hanging ends. That process is more error prone than homologous recombination because the hanging end is allowed to join any random, matching strand in the cell. Nonhomologous end joining often leads to mutation, or an incorrect DNA sequence. Church and his colleagues assessed both methods in their experiments.

After explaining their technology, Church and his colleagues discuss how they tested RNA-guided Cas 9 gene editing using homologous recombination. The authors explain that they tested RNA-guided Cas 9 gene editing on an inserted gene meaning it was artificially placed into the cell. They then detail their experiment, which used a human embryonic kidney cell line and Green Fluorescent Protein, or GFP. Cell lines are cultures of cells that all have the same genetic makeup. GFP, discovered in jellyfish, exhibits green fluorescence when exposed to blue to UV light. GFP is a useful marker because any cell with a functioning GFP gene will express GFP and exhibit the green fluorescence.

The authors describe their mutated GFP gene as broken, meaning that cells with their GFP gene could not exhibit green fluorescence. Therefore, Church and his team started with human cells with a broken GFP gene that could not fluoresce green. As detailed in the article, they then planned to deploy their RNA-guided Cas 9 technology to repair the non-functioning GFP gene. That meant that if their experiment was successful, cells would fluoresce green, indicating successful genomic editing. With that, Church and his co-authors establish the parameters of their experiment and the indicators of success.

Church and his colleagues discuss their process for attempting to repair the non-functioning GFP protein with the RNA-guided Cas 9 technology. In their experiment, the researchers used two guide RNAs called T1 and T2. Each guide RNA had slight variations in their sequence, but both targeted the GFP gene region. The donor sequences the authors used had the correct sequence for GFP for insertion into the genome of the human cell. To outline how the mutated GFP is repaired in the cell, the authors explain that the guide RNA targets the GFP mutated site in the human genome. Once the guide RNA identifies the correct site, Cas 9 cuts the DNA and the donor sequence replaces the cut-out DNA strand. The gene is repaired by homologous recombination because the donor sequence for the proper GFP gene replaces the faulty gene. Church and his colleagues describe examining the number of cells exhibiting green fluorescence to determine the success of the guide RNA and Cas 9. The researchers sorted cells based on fluorescence. Church and his colleagues note that the T1 guide RNA had a correction rate of three percent, meaning that three percent of cells targeted with that RNA fluoresced green. The T2 guide had a correction rate of eight percent. Church and his colleagues conclude that that T2 was a better guide RNA than T1.

In their article, the authors also compare those correction rates to correction rates of more complicated methods of genomic editing called TALENs. TALENs use different proteins to cut DNA and insert desired sequences. Church and his team report that they could detect green fluorescence after twenty hours for cells treated with Cas 9 and after forty hours for cells treated with TALENs. Thus, the authors argue that the Cas 9 technology showed a faster rate of gene editing in the human cell lines. They also discuss that they observed higher toxicity or cell death in using TALENs as opposed to the Cas 9 technology.
Church and his colleagues also discuss some other benefits of their RNA-guided Cas 9 technology. They first show that their designed guide RNAs were sequence-specific, meaning that the guide RNA sequence must match the target DNA sequence to bind to it and proceed with gene editing. To demonstrate that their guide RNAs were sequence-specific, the authors outline how they mutated a target DNA sequence in the human cells so that the guide RNA would not match it and tested its effect on the Cas 9/guide RNA system. They report that when the sequence was mutated, no cells exhibited green fluorescence. The authors explain that when guide RNA sequences do not match their target DNA sequences, no editing occurs. That result confirmed that guide RNAs target specific DNA sequences to allow Cas 9 to affect them.

The authors also highlight that it is possible to use multiple guide RNAs at once in their system. Multiplexing in gene editing is the ability to edit different genes at the same time. To show that multiplexing was possible, the authors discuss how they designed two new guide RNAs that targeted different sites in the human genome. The authors discuss treating the cells with two and three different guide RNAs at once and report that Cas 9 could edit the three different gene sites at once.

Church and his colleagues report one other capability of their RNA-guided Cas 9 technology, genomic editing of a native gene in human cell lines. Native genes are naturally found in humans, as opposed to genes like GFP that are inserted by researchers. The authors tested that technique using nonhomologous end joining and homologous recombination repair. The authors note that to test nonhomologous end joining, they did not use a donor sequence, meaning that the cell had to provide the new DNA sequence. The researchers explain that they targeted a specific gene in three kinds of cells, cutting out those genes with the Cas 9 system and letting the cells repair the gaps in the DNA. The authors used DNA sequencing to determine whether the edits were successful, looking specifically for the correct DNA sequence in the genome. The authors state that CRISPR/cas 9 was successful for cutting native genes in human cell lines.

To test the same technique using homologous recombination repair, Church and his team detail the same protocol with the addition of including donor sequences to repair the DNA. Church and his colleagues point out that they designed a double-stranded DNA donor sequence for a specific segment of DNA. The authors discuss treating human cell lines with Cas 9, guide RNA, and the donor sequence. They note that successful homologous recombination was confirmed with DNA sequencing.

The authors conclude "RNA-Guided Human Genome" with the claim that they have shown RNA-guided Cas 9 as a robust system for gene editing. The authors also claim that RNA-guided Cas 9 gene editing is superior to TALENs, an alternate method of gene editing. To help other scientists use RNA-guided Cas 9 for gene editing, Church and his colleagues point out that they created a database of 190,000 guide RNA sequences specific to human genes. Church and his colleagues published their resource online to provide a human genome-wide reference for identifying Cas 9 target sites. Church and his colleagues' publication has prompted scientists to use RNA-guided Cas 9 for gene editing in hundreds of organisms. As of July 2017, over 3,000 scientists and researchers have cited Church and his colleagues' publication.
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**Subject**


**Topic**

Publications [23]

**Publisher**

Arizona State University. School of Life Sciences. Center for Biology and Society. Embryo Project Encyclopedia.