

Science Education Collection

Genome Editing

URL: <https://www.jove.com/science-education/5554>

Abstract

A well-established technique for modifying specific sequences in the genome is gene targeting by homologous recombination, but this method can be laborious and only works in certain organisms. Recent advances have led to the development of “genome editing”, which works by inducing double-strand breaks in DNA using engineered nuclease enzymes guided to target genomic sites by either proteins or RNAs that recognize specific sequences. When a cell attempts to repair this damage, mutations can be introduced into the targeted DNA region.

In this video, JoVE explains the principles behind genome editing, emphasizing how this technique relates to DNA repair mechanisms. Then, three major genome editing methods—zinc finger nucleases, TALENs, and the CRISPR-Cas9 system—are reviewed, followed by a protocol for using CRISPR to create targeted genetic changes in mammalian cells. Finally, we discuss some current research that applies genome editing to alter the genetic material in model organisms or cultured cells.

Transcript

Genome editing comprises techniques with which researchers can “edit” or change a specific DNA sequence. These methods rely on the creation of small “cuts” in DNA, which cells attempt to repair, often incompletely. In this manner, scientists can induce mutations in targeted sequences with greater efficiency compared to classical gene targeting.

In this video, we will review the principles behind three genome editing techniques, and discuss a generalized protocol for one of them, the CRISPR-Cas9 system. We will then explore some applications of these methods.

First, let's look at the principles behind genome editing.

The classical method for introducing genetic changes to specific sequences in the genome is gene targeting by homologous recombination, where the incorporation of homologous sequences into the targeting construct leads to the “switching out” of the endogenous gene for an altered version.

Like gene targeting, genome editing can target changes to specific DNA regions, but it can do so with much higher efficiency, establishing the desired mutations in more cells in any given experiment.

All genome editing methods rely on a cell's ability to repair double-strand breaks made to its DNA by endonucleases. This damage may be repaired by nonhomologous end joining or NHEJ, where broken DNA ends are resealed directly; or by homology-directed repair or HDR, where the damage is fixed by copying from a homologous template. NHEJ is not usually perfect, and could result in several bases being deleted or added to the repaired DNA, thus mutating the target sequence. On the other hand, HDR allows researchers to add in a template to direct specific changes to the target site.

Three major genome editing techniques have been developed and popularized over the last few years.

In one method, researchers fuse DNA-binding “zinc finger” domains of certain transcription factors to the DNA-cleaving domain of the *FokI* endonuclease. As each zinc finger domain recognizes a specific nucleotide triplet, these domains can be artificially linked together to engineer zinc finger nucleases, or ZFNs, that target unique DNA sequences.

Similarly, nucleases called “TALENs” join *FokI* to the variable DNA-binding domains of bacterial proteins called transcription activator-like effectors, or TALEs. Each TALE domain recognizes a single, unique DNA base, giving TALENs their sequence specificity.

Finally, the CRISPR-Cas9 system uses components of a prokaryotic immune system that normally protects its host against incursion by foreign genetic materials, like those in viruses or plasmids. In this system, pieces of invading foreign DNA called “protospacers” are incorporated into a CRISPR locus, which is then transcribed and processed by protein-RNA machinery into small RNAs called crRNAs.

Scientists are harnessing the CRISPR machinery for genomic editing purposes by designing crRNA-like guide sequences known as “single guide” or sgRNA, which can be used to target Cas9 to almost any desired genetic sequence in mammalian cells, or other systems of interest. The simple customizability of the RNA sequence-based CRISPR-Cas9 system provides distinct advantages over protein-based genome editing methods like ZFNs and TALENs.

Now that you've learned about the principles behind genome editing, let's review a typical protocol of using CRISPR to create targeted genetic changes in mammalian cells.

First, a genomic location to be edited is chosen. This region must be short—approximately 20 base pairs in size—and possess a 3-basepair long “protospacer adjacent motif” or “PAM” at its 3' end. The PAM is required for Cas9 to recognize and cleave the target DNA region, and the exact sequence is specific to the organism-of-origin of the Cas9 being used. Once a potential site has been identified, it should be searched against the genome sequence to ensure that similar sites are not found elsewhere in the genome, and thus no off-target genomic regions will be edited.

To synthesize the CRISPR guide sequence, two oligonucleotides are generated, one identical and one complementary to the target sequence. Extra sequences are included on their 5' ends for compatibility with the sgRNA vectors. These oligonucleotides are then mixed together, denatured, and then annealed.

Next, a plasmid containing an sgRNA “scaffold” is cut with the appropriate restriction enzyme, mixed with the synthesized CRISPR guide sequence, and incubated with ligase enzyme to create the sgRNA construct. The plasmid can then be transformed into bacteria and cultured for amplification. Once purified from bacteria, the CRISPR construct is co-transfected with a construct encoding Cas9 into the cells whose genome is to be edited. These transfected cells are cultured to form clonal colonies.

Genomic DNA can be isolated from each colony and analyzed by PCR or sequencing to screen for those in which the CRISPR system has produced the desired mutations.

Let's now look at some ways scientists are using genome editing techniques.

Many researchers use genome editing to introduce reporter sequences into specific loci. In this experiment, ZFNs were used to insert green fluorescent protein into a gene that is involved in neuron development and survival. Researchers confirmed that they correctly targeted this gene by directing stem cells to differentiate into neurons, and performing double immunofluorescence for GFP and neuronal markers.

Genome editing has also made it much easier to generate “knockout” organisms in which a gene of interest is rendered non-functional. Here, researchers sought to create knockout mice by injecting embryos with mRNA encoding TALENs that target specific genes. Subsequent endonuclease treatment of DNA from TALEN-treated mice allowed researchers to identify animals with mutations in both copies of the targeted gene.

Finally, genome editing can be used to create large genetic deletions by causing two double-strand breaks in the same chromosomal region. These large deletions might be necessary when the function of a gene or genetic element cannot be knocked out by the small deletions created with conventional genome editing protocols. Here, researchers co-electroporated mouse cancer cells with a GFP construct and two CRISPR constructs targeting different areas within a cancer-driving gene. Cells that were successfully transfected, which would thus emit GFP signal, were isolated by fluorescence-activated cell sorting, and subsequent PCR and sequencing identified cells in which the DNA region between the two CRISPR-targeted sites was deleted.

You've just watched JoVE's video on genome editing. Here, we've reviewed the principles behind ZFNs, TALENs, and CRISPR, explored a general CRISPR-Cas9 protocol, and discussed how researchers are using genome editing techniques today. As always, thanks for watching!