Title: 5554-Genome Editing

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.

Application Videos:

1. Zinc-finger Nuclease Enhanced Gene Targeting in Human Embryonic Stem Cells **(Thumbnail 51764@7:22-image of stem cell colony)**

Description: By taking advantage of a DNA repair mechanism called homology-directed repair, genome editing can be used to introduce new sequences into an organism’s genome. This video discusses a protocol that employs ZFNs to insert a fluorescent reporter sequence into a specific gene in human embryonic stem cells, allowing researchers to track when and under what circumstances this gene is expressed.

2. Mouse Genome Engineering Using Designer Nucleases **(Thumbnail 50930@0:22-TALEN schematic)**

Description: An important application of targeted gene modification techniques is to create knockout strains of organisms, which do not express any functional products from a specific gene. Here, scientists demonstrate how to use TALENs to generate knockout mice, a process that involves injecting mouse embryos with mRNA encoding for these engineered nucleases. By analyzing DNA from the resulting mice, scientists were able to isolate animals in which the TALENs successfully altered both copies of a gene of interest.

3. Generation of Genomic Deletions in Mammalian Cell Lines via CRISPR/Cas9 **(Thumbnail 52118@2:03-talent introducing liquid into electroporation cuvette)**

Description: Genome editing can be adapted to create large deletions at a specific genomic site, a method some researchers use to ensure that their gene of interest is knocked out. The authors of this article employed the CRISPR-Cas9 system to create large deletions in a specific protein-encoding mouse gene, *Pim1*. This was accomplished by designing and introducing two CRISPR constructs, each targeting a different region in *Pim1*, into mouse cells, which resulted in the sequence between these sites getting removed when the cell attempted to repair this damage by a process called nonhomologous end joining.

4. Production of Apolipoprotein C-III Knockout Rabbits Using Zinc Finger Nucleases **(Thumbnail 50957@6:00-rabbit embryo getting microinjected)**

Description: One of the benefits of genome editing is that it can be performed in model organisms in which classical gene targeting does not work. Researchers here used ZFNs to knockout a gene in rabbits, *APOC3*, which has been associated with cardiovascular disease.

5. Genome Editing with CompoZr Custom Zinc Finger Nucleases (ZFNs) **(Thumbnail 3304@3:34-machine introducing liquid into several black plates [as part of ZFN design])**

Description: In this video, researchers used a technique, termed the Cel-1 assay, to evaluate the efficiency of ZFNs. DNA was collected from a ZFN-treated leukemia cell line, denatured, and then re-annealed, resulting in two different types of DNA molecules: those with two strands of the same type of DNA (either normal or mutated), and those composed of one mutant and one normal strand, called “heteroduplexes.” By treating DNA with an enzyme that only cuts heteroduplex molecules, researchers were able to determine the proportion of leukemia cells in the ZFN-treated population that have the desired mutation.

Related Science Education Videos:

5036 – An Introduction to Working in the Hood

5074 – Molecular Cloning

5327 – Genetic Engineering of Model Organisms

5552 – Introduction to Genetic Engineering