

Science Education Collection

Genetic Engineering of Model Organisms

URL: <https://www.jove.com/science-education/5327>

Abstract

Transgenesis, or the use of genetic engineering to alter gene expression, is widely used in the field of developmental biology. Scientists use a number of approaches to alter the function of genes to understand their roles in developmental processes. This includes replacement of a gene with a nonfunctional copy, or adding a visualizable tag to a gene that allows the resultant fusion protein to be tracked throughout development.

In this video, the viewers will learn about the principles behind transgenesis, as well as the basic steps for introducing genetic constructs into an animal and targeting genes of interest. This is followed by the discussion of a protocol to create knockout mice. Lastly, some specific applications of transgenic technologies in the field of developmental biology will be reviewed.

Transcript

Genetic engineering is a valuable tool used to modify genomes of model organisms in a process known as transgenesis. In developmental biology, this approach is often used to express modified genes that can be visualized in living tissues. Alternatively, genetic engineering can be used to prevent or disrupt protein expression to study the developmental function of specific genes.

This video will summarize the principles behind this technology, review some genetic engineering procedures, and highlight ways that these techniques are used in the lab.

To begin, let's explore some important concepts underlying transgenesis. This involves insertion of DNA into the genome of a model organism. There are a number of approaches depending on the study goal.

First, addition of an altered gene might reveal functional or morphological changes due to a mutation. Another method is to put in additional copies of the unaltered wild-type gene to study the effects of overexpression, which can often be just as damaging as a mutation. A different approach is to insert a fusion protein that contains a visualizable tag, such as green fluorescent protein, to track the location and timing of gene expression in live animals.

The segment of DNA that will be inserted into the genome must be carefully designed to produce the desired expression patterns and outcomes. The promoter, which is a sequence element that dictates when and where a gene is expressed, is a crucial component. Certain promoters are ubiquitously expressed throughout almost all tissues, while others are only active in specific tissues. Inducible promoters, which are activated by chemical administration or exposure to high temperatures, can also be used to control timing of gene expression.

To be stably expressed in tissues, a transgene must first integrate into the genome. To accomplish this, transgenes can include flanking DNA sequences that match areas of the organism's genome. This allows the transgene to integrate with the host DNA through a process known as homologous recombination. Alternatively, in some species special elements called transposons can make transgenesis more efficient by including recognition sites for the enzyme transposase, which catalyzes random insertion of the transgene into the genome.

Now that you know some of the basics of transgene design, let's review how to make a transgenic animal. To make the transgene construct, start by amplifying the gene of interest using PCR. This amplified region is then cloned into a vector, which is a piece of DNA that can carry the transgene into cells. Vectors typically contain elements that allow efficient transgene amplification using bacteria, such as *E. coli*. After this amplification step, the vector is purified from the bacterial culture.

Transgenic animals are made by injecting purified DNA into embryos. In fish and frogs, constructs are usually injected directly into the yolk or cytoplasm of one-cell stage embryos. For transposon-mediated transgenesis, a transcript encoding the transposase enzyme is added to the injection mix.

In mice, transgenesis can be accomplished by manipulation of newly fertilized eggs in which the sperm and egg pronuclei have not yet fused. The construct is injected directly into the larger pronucleus, where it may integrate into the genome as the cell divides. The eggs must then be transplanted into the uterus of a pseudopregnant female for development.

Transgenesis efficiency varies, so animals must be screened to identify progeny in which the construct has successfully integrated into the genome. This can be done by looking for a fluorescent tag that was inserted for easy identification, or through molecular analyses such as PCR of genomic DNA isolated from small tissue pieces.

A second approach to genetic engineering focuses on specific gene targeting to disrupt gene function. There are multiple approaches to achieve this goal. One relatively new method, known as genome editing, takes advantage of sequence-specific enzymes called nucleases, which cut the DNA backbone and cause mutations in genes as the DNA is repaired.

Another targeting method involves the use of homologous recombination to replace a gene with either foreign DNA or a copy of the gene flanked by recognition sequences for enzymes known as recombinases. When the recombinases are present, the flanked sequence will be excised from the genome. This is known as a conditional knockout, and control of gene excision can be achieved by expressing the enzyme in specific tissues or at certain time points.

Let's review a general procedure for generating knockout mice by homologous recombination. Here, a construct must be prepared in which part of the genomic DNA sequence is replaced with foreign DNA. This DNA often encodes another gene, such as for antibiotic resistance, which provides a way to select successfully modified cells in later steps.

To begin the procedure, embryonic stem cells are collected from the inner cell mass of an early mouse embryo known as a blastocyst. The linearized construct is then delivered into the stem cells via electroporation, in which electrical pulses generate transient pores in the cell membrane. The cells are then allowed to incubate in the presence of an antibiotic to eliminate cells without the transgene.

After this selection step, the stem cells can be injected into another mouse embryo at the blastocyst stage. The embryos are then transferred to the uterus of a female mouse to continue development. The resulting pups will be chimeras, which are composed of both wild-type and knockout cells. Some chimeras will have knockout cells within their germline, which will transmit the disrupted gene when they are bred, which will then establish a new knockout line.

You have learned the basics of genetic engineering of developmental models, so now let's look at some practical applications.

Developmental studies often use fluorescently tagged proteins to identify cells and study their development. Using tissue-specific promoters, transgenic organisms can be engineered to express fluorescent proteins in specific cells, like the neural crest. Using advanced imaging techniques, the fluorescent cells can be imaged in real time, allowing researchers to directly visualize complex developmental events.

Another important use of genetic engineering is to study specific genes and their role in disease phenotypes. Here, targeted mutations are introduced into a specific mouse gene using nucleases, such as TALENs. PCR shows whether the mouse has zero, one, or two copies of the gene mutated. The embryos carrying two mutant copies can now be studied in detail to determine the developmental function of the gene.

Using conditional knockouts, scientists can determine the function of a gene within a restricted set of cells. Here, a loxP-flanked gene was expressed throughout the entire embryo, but Cre was expressed in endothelial cells only, causing a gene deletion in the heart and blood vessels. This tissue-specific knockout resulted in a measureable change in embryonic heart rate, and illustrates how to test the localized role of a gene without changing the entire organism.

You've just watched JoVE's introduction to transgenic technology. These techniques help you understand the basics of genetic engineering, some of the methods that are involved, and how it is applied in everyday science. Genetic engineering can be widely applied across many organisms, and will continue to be an important tool for studying and understanding the role of genetics in developmental diseases, as well as those that appear during adulthood. Thanks for watching!