Title: Introduction to Genetic Engineering

Abstract: Genetic engineering – the process of purposefully altering an organism’s DNA – has been used to create powerful research tools and model organisms, and has also seen many agricultural applications. However, in order to engineer traits to tackle complex agricultural problems such as stress tolerance, or to realize the promise of gene therapy for treating human diseases, further advances in the field are still needed. Important considerations include the safe and efficient delivery of genetic constructs into cells or organisms, and the establishment of the desired modification in an organism’s genome with the least “off-target” effects.

JoVE’s Overview of Genetic Engineering will present a history of the field, highlighting the discoveries that confirmed DNA as the genetic material and led to the development of tools to modify DNA. Key questions that must be answered in order to improve the process of genetic engineering will then be introduced, along with various tools used by genetic engineers. Finally, we will survey several applications demonstrating the types of experimental questions and strategies in the field today.

Application videos:

1. Transient Gene Expression in Tobacco using Gibson Assembly and the Gene Gun **(51234 Thumbnail @2:15 – Gibson Assembly schematic)**

Description: Classical molecular cloning uses restriction enzymes to create compatible ends between different DNA fragments that are then ligated together into a completed construct. On the other hand, a recently developed technique known as Gibson assembly is based on the annealing between complementary sequences in the fragments, thus removing the need for restriction endonucleases. In this article, researchers used Gibson assembly to generate genetic constructs that express proteins with specific subcellular localization in tobacco plants. The constructs were transfected into cells with a gene gun – a method known as biolistics – and subcellular localization was visualized with fluorescence microscopy.

2. An Orthotopic Bladder Cancer Model for Gene Delivery Studies**(50181 Thumbnail @0:24 – cartoon illustrating viral vector and mouse bladder)**

Description: In this video, researchers utilize an orthotopic bladder cancer model, where human bladder cancer cells are used to establish tumors in the bladders of live mice. This model allows researchers to test the safety and efficacy of various gene delivery vectors in a setting that accurately mimics the human disease state, facilitating the development of gene therapy. Cells and viral vectors are both delivered to the bladder via catheterization, and a luciferase reporter, which emits a bioluminescence signal, is used to visualize gene delivery to tumor cells.

3. The Logic, Experimental Steps, and Potential of Heterologous Natural Product Biosynthesis Featuring the Complex Antibiotic Erythromycin A Produced Through *E. coli* **(4346 Thumbnail @0:47 – schematic of the six transgene constructs)**

Description: Currently, genetic engineering often involves making modifications to a small number of individual genes. However, the future of this field points toward more complex scenarios requiring simultaneous modification of multiple gene targets, perhaps whole biological pathways. In this article, researchers reconstituted the entire biosynthetic pathway for the antibiotic erythromycin A, originally found in *S. erythraea* (a slow-growing bacteria with limited ability for genetic manipulation), in *E. coli* (a fast-growing, easily manipulated bacteria). This involved isolating more than 20 genes and organizing them into operon structures, where multiple genes were placed under the control of the same regulatory elements, for coordinated expression in *E. coli*. Expression of these genes and the production of functional antibiotic were then verified, demonstrating functional transfer of this pathway.

4. Therapeutic Gene Delivery and Transfection in Human Pancreatic Cancer Cells using Epidermal Growth Factor Receptor-targeted Gelatin Nanoparticles **(3612 Thumbnail @6:54 – Fluorescent image showing transfection efficiency)**

Description: For delivering genetic constructs to cells, transfection efficiency is often the most desired attribute. However, excessive cytotoxicity reduces the efficacy of gene delivery, and when attempting to deliver constructs to human patients for gene therapy, safety and minimal toxicity become paramount considerations. Here, scientists present a method for generating gelatin nanoparticles, which demonstrate reduced cytotoxicity compared to other transfection reagents, and specifically targeting the particles to the epidermal growth factor receptor that is overexpressed in some types of tumor cells, thus minimizing any effect on healthy, non-target cells.

5. Regioselective Biolistic Targeting in Organotypic Brain Slices Using a Modified Gene Gun **(52148 Thumbnail @6:00 – Image of red-colored neurons)**

Description: Biolistics is the process of “shooting” DNA- or dye-labeled gold particles into cells or tissues of interest. In this protocol, biolistic transfection was used to label terminally differentiated neurons in anatomically defined regions of organotypic brain slices, which could be maintained in tissue culture while continuing to exhibit functional neural connections. This allowed scientists to study the effects of individual genes on cells in brain regions of interest.

Related Videos

5161 – Introducing Experimental Agents into the Mouse

5215 – Neuronal Transfection Methods

5325 – An Introduction to Developmental Genetics

5553 – Recombineering and Gene Targeting