Title: 5542-Genetic Screens

Abstract: Genetic screens are critical tools for defining gene function and understanding gene interactions. Screens typically involve mutating genes and then assessing the affected organisms for phenotypes of interest. The process can be “forward”, where mutations are generated randomly to identify unknown genes responsible for the phenotypes, or it can be “reverse”, where specific genes are targeted for mutation to observe what phenotypes are produced.

Here, JoVE reviews various types of genetic screens, including those that depend on either loss-of-function or gain-of-function mutations, which respectively decrease or increase the activity of genes. We then explore general protocols for forward and reverse screens in a popular model organism, the nematode worm. Finally, we highlight how screens are applied in research today, for example to better understand gene interactions that may contribute to neurodegenerative diseases.

Application Videos:

1. A High-content Imaging Workflow to Study Grb2 Signaling Complexes by Expression Cloning **(Thumbnail 4382@5:56-remove text overlay, clip of cell “dispenser”/black machine with clear tubes emerging from its left side)**

Description: This video presents a protocol for an overexpression screen that can be used to evaluate protein interactions. Fluorescently labeled Grb2 protein, which is involved in cell signaling, was expressed in a human cell line. A cDNA library, containing all the protein-coding sequences in the genome, was then transformed into these cells and overexpressed, and specimens with altered Grb2 localization were examined to identify proteins that could associate with this signaling molecule.

2. Competitive Genomic Screens of Barcoded Yeast Libraries **(Thumbnail 2864@2:32-plate with yeast colonies)**

Description: Genetic screens can also be applied to investigate how drugs affect organisms with different genotypes. This study used a library of mutant yeast strains that were generated by random transposon insertion, each identified by a distinct “barcode” sequence in its DNA. The yeast strains were pooled and then exposed to an antifungal drug. By isolating DNA from treated yeast and assessing the levels of each barcode, researchers identified mutant strains that were sensitive to the antifungal and demonstrated poor growth in its presence.

3. High Content Screening in Neurodegenerative Diseases **(Thumbnail 3452@6:46-plate being manipulated by machine/being prepared for imaging)**

Description: To gain insight into neurodegenerative illnesses, the authors of this article used lentiviral vectors to deliver a library encoding short-hairpin RNAs into a human neural cell line, knocking down the expression of hundreds of genes. These cells were then directed to differentiate into neurons, and researchers screened for changes in neuron survival and the expression of DJ1, a protein associated with Parkinson’s disease.

4. High-throughput Yeast Plasmid Overexpression Screen **(Thumbnail 2836@6:19-talent placing 96-well plate into machine)**

Description: Researchers can combine different types of genetic screens to better understand gene interactions and how such interactions contribute to diseases. Here, a screen that employed the principles of modifier and overexpression screens was performed in yeast. A library encoding all protein-coding sequences in the yeast genome was overexpressed in a strain already transformed to express TDP-43, a protein implicated in Lou Gehrig’s disease. As TDP-43 is toxic at high levels in yeast, scientists could determine whether the overexpressed genes suppressed or enhanced the effects of TDP-43 by assessing growth and survival of the doubly-transformed yeast.

5. Fluorescence-microscopy Screening and Next-generation Sequencing: Useful Tools for the Identification of Genes Involved in Organelle Integrity **(Thumbnail 3809@4:04-talent planting *Arabidopsis* seedling)**

Description: This video presents a forward genetic screen in *Arabidopsis* plants, whose seeds were treated with EMS, a chemical mutagen. The plant line used here had been engineered such that the endoplasmic reticulum (ER), a network of membranous tubes for intracellular transport, was fluorescently labeled. Once the mutated plants had matured, researchers evaluated plants for abnormal ER morphology, and then collected and analyzed DNA from their progeny. Using this protocol, scientists were able to identify genes involved in ER maintenance.

Related Science Education Videos:

5083 – Yeast Transformation and Cloning

5105 – RNAi in *C. elegans*

5215 – Neuronal Transfection Methods

5540 – Genetics of Individuals and Populations