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Yeast Transformation and Cloning

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Abstract

S. cerevisiae are unicellular eukaryotes that are a commonly-used model organism in biological research. In the course of their work, yeast researchers rely upon the fundamental technique of transformation (the uptake of foreign DNA by the cell) to control gene expression, induce genetic deletions, express recombinant proteins, and label subcellular structures.

This video provides an overview of how and why yeast transformation is carried out in the lab. The important features of yeast plasmids will be presented, along with the procedure required to prepare yeast cells to incorporate new plasmids. The presentation also includes a step-by-step protocol for the lithium acetate method of yeast transformation. Finally, examples of the many applications of this essential technique will be provided.

Transcript

Yeast or *Saccharomyces cerevisiae*, is a widespread simple eukaryotic model organism used in the study of genetics and cell biology that can give insights into human cellular processes. This video discusses transformation - the uptake of foreign DNA by the yeast cell. It will introduce yeast plasmids, how to prepare yeast cells for transformation, a step-by-step transformation procedure, and will provide some applications of this fundamental technique.

Before we talk about the transformation of yeast, let's first discuss a type of DNA used in transformation: the plasmid. A plasmid is a small, circular, double-stranded DNA that can supercoil so it can easily pass through pores in a cell membrane.

Plasmids contain a multiple cloning site or MCS where restriction endonucleases, AKA restriction enzymes, can cut DNA. DNA fragments of interest cut with the same enzymes can then be ligated into the MCS. Plasmids also contain an origin of replication or ORI that signals to the cell where replication should begin. In addition, plasmids have a selectable marker, which allows the yeast cells that contain the plasmid to grow under specific environmental conditions. Yeast that don't successfully incorporate the plasmid will not survive in media containing the selectable marker. The selectable markers can encode for genes that enable drug-resistance or genes that encode enzymes that enable a yeast strain to synthesize amino acids that they otherwise cannot produce.

Shuttle vectors are plasmids that can replicate in more than one host species. For instance, a plasmid from *E. coli* can grow in yeast. Yeast Plasmids can be non-integrating or integrating, meaning that the plasmid either combines with the genomic DNA or remains independent.

There are five different general types of plasmids or vectors that are used in yeast. The two that are used most often in the transformation of yeast are the yeast episomal plasmid or YEp and the yeast centromeric plasmid or YCp. Both of these types of vectors contain an autonomous replication sequence or ARS. The ARS contains the origin of replication and allows for extrachromosomal replication in yeast.

There are several different procedures that can be used to transform yeast which include the spheroplast method, electroporation, and lithium acetate-mediated transformation. For this video we will focus on the lithium acetate procedure.

In this transformation method positively-charged lithium cations neutralize charges on the cell membrane and plasmid DNA. Single-stranded DNA - added to the transformation mixture - binds to the cell wall of the yeast and leaves the plasmid DNA available for uptake by the yeast cells. The exposure to a sudden increase in temperature, or heat shock creates a pressure difference between the inside and outside of the cell creating pores that plasmid DNA can pass through. When the temperature is decreased, the yeast cell wall will reform and transformation is complete.

Let's begin with a step-by-step procedure of how to prepare yeast for transformation. Yeast cells must be prepared by first picking a colony from an agar plate and amplifying the colony in yeast extract peptone dextrose medium, abbreviated YPD - a complete medium for yeast growth.

After the colony is picked from a plate and placed into YPD medium, the culture is incubated overnight at 30 °C with agitation on a shaker or roller apparatus, like you see here. The yeast cells are pelleted by centrifugation and the supernatant is removed. The pelleted cells are resuspended with the desired buffer or sterile water. These competent prepared yeast cells will be used in the transformation procedure.

Once yeast cells have been prepared, transformation can be carried out by first preparing the transformation mixture.

This reagent mixture should include: sterile distilled water; a solution of 50% polyethylene glycol or PEG, 1M lithium acetate, 10 mg/ml solution of single-stranded DNA, plasmid DNA and competent yeast cells. The exact proportions of each solution should be calculated before beginning the experiment by consulting your laboratory's standard protocol for yeast transformation.

The mixture is then incubated at 30 °C for 30 minutes with shaking. The solution should be mixed, not vortexed, to ensure the yeast cells do not break apart.

The cells are heat-shocked by placement in a 42 °C water bath for 15 minutes followed by cooling on ice for 2 minutes. The cells are then harvested by centrifugation.

Cells are resuspended in double-distilled water and are plated on agar plates that will select for the desired transformants. Transformation plates are then incubated at 30 °C for two to four days until colonies form.

Transformation procedures should always include positive and negative control plates until they are optimized. The positive control should be a yeast cell suspension with plasmid DNA on a YPD plate that does not contain any selectable marker. This shows that the cells are healthy following the transformation procedure. The negative control plate should be a yeast cell suspension on an appropriate selection plate, such as one that contains antibiotics. The plate should have no colonies and shows that there is no contamination.

There are a myriad of different applications for yeast transformation. One application of transformed yeast is to use a yeast-two hybrid system to identify proteins that interact with your protein of interest, or the bait protein. When a plasmid from a library of candidate binding partners, or prey proteins, are transformed and there is an interaction, a transcription factor is released that will activate a reporter gene, such as Beta-galactosidase. The reporter gene will turn colonies that have an interaction blue when on plates that contain X-gal — a substrate for beta-galactosidase.

Multiple deletions can be engineered into yeast through a technique using sexual cycling. Haploid cells that contain a reporter and deleted locus are combined into one cell through random assortment or meiotic recombination. Selectable markers are used to select for yeast that have successfully incorporated the deletions. In this case, flow cytometry is used to select for cells that express GFP.

Yeast can be transformed with proteins that are fluorescently labeled to view the effect of mutations on protein-protein interactions. This video-article used fluorescent microscopy to study the effects of different mutations on protein-protein interactions essential for endocytosis.

You've just watched JoVEs video on yeast transformation. You should now understand the basic aspects of a plasmid, how to prepare yeast cells for transformation and how to perform the transformation procedure. As always, thanks for watching!