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Isolating Nucleic Acids from Yeast

URL: <https://www.jove.com/science-education/5096>

Abstract

One of the many advantages to using yeast as a model system is that large quantities of biomacromolecules, including nucleic acids (DNA and RNA), can be purified from the cultured cells.

This video will address the steps required to carry out nucleic acid extraction. We will begin by briefly outlining the growth and harvest, and lysis of yeast cells, which are the initial steps common to the isolation of all biomacromolecules. Next, we will discuss two unique purification methods for the separation of nucleic acids: column binding and phase separation. Additionally, we will demonstrate several ways in which these methods are applied in the laboratory, including the preparation of nucleic acids for molecular biology techniques such as PCR and southern blotting, quantification of gene expression in response to environmental stimuli, and purification of large amounts of recombinant proteins.

Transcript

Today we will be showing you how to purify nucleic acids from *Saccharomyces cerevisiae*, also known as baker's yeast. Nucleic acids can include DNA or RNA. This video will discuss the isolation of these molecules from yeast cells by phase separation and chromatography.

Though many different methods exist to purify nucleic acids from yeast, most of them share the same initial steps.

Yeast cells are first propagated by selecting a single colony from a plate and inoculating into YPD media. The mixture should be grown overnight at 30 °C in a shaking or rotating incubator.

Yeast cells should be harvested in the mid-log phase of growth to optimize yield. Yeast in the log phase of growth will usually have an optical density or OD value of 0.5-1 when measured at a wavelength of 600 nm. Once cells have reached the appropriate optical density, they are centrifuged to form a pellet, and resuspended in lysis buffer so that cells are broken open.

One of the most challenging aspects of isolating nucleic acids from yeast is disrupting its tough cell walls. Cell walls can be destroyed with a combination of enzymatic and physical techniques. The destruction of the cell wall causes yeast to form spheroid cells called spheroplasts that can be lysed via standard cell lysis techniques.

Spheroplasts are typically lysed with chemical detergents such as sodium dodecyl sulfate or SDS, which lyse cellular membranes. Cells can also be homogenized. For instance, glass beads can be added to cells and cells homogenized by vortexing. Or cell walls can be disrupted with ultra-high frequency sound, using a sonicator, to aid in the lysis process.

As mentioned all nucleic acid purification procedures done in yeast will have similar steps for growing up, harvesting, and lysing yeast cells, however once cells are lysed, several different methods can be used to isolate nucleic acids. Nucleic acids can be purified through column binding or phase separation.

DNA and RNA are best-isolated using silica column binding. Nucleic acids will bind to the column through anion exchange and can be eluted from the column once separated from other cellular components.

Phase separation uses the principle that solutions with different properties can be used to purify or concentrate certain proteins or nucleic acids based on their solubility. The addition of chloroform differentiates a slurry of cell components into two different phases, the aqueous and organic. The organic phase contain proteins while the aqueous phase contains nucleic acids. The DNA can then be precipitated from the organic phase with the addition of ethanol.

Nucleic acids can be isolated from yeast using a column binding protocol. First, cells are grown up and harvested by centrifugation.

The supernatant is removed and discarded while the cell pellet is resuspended in enzyme-containing buffer, vortexed, and incubated until cell walls are digested. Cell wall digestion and spheroplast formation can be verified with microscopy when optimizing enzyme treatment. After cell wall digestion, lysis buffer is added to the cells and the mixture is vortexed.

The lysed cells should be centrifuged to clarify the mixture of debris, leaving DNA and small particulates and soluble proteins in the supernatant. The supernatant is loaded onto a silica column and nucleic acids are then allowed to bind following centrifugation, which will remove a bulk of the soluble impurities.

Washing steps are performed with ethanol or high salt buffer to remove residual impurities from the bound nucleic acids. Finally, the DNA or RNA is eluted with water or a buffer low in salt. Be sure to use a buffer or water that is free of the enzymes DNase and RNase.

Nucleic acids isolated from yeast have a variety of uses depending on your specific experimental goal. DNA isolated from yeast can be used for a number of different molecular biology techniques including: PCR, southern blotting, or restriction enzyme digestion.

Changes in gene expression can be identified by a process known as microarray analysis, which uses gene arrays like this one.

If we have two yeast cultures, one exposed to hydrogen peroxide and one a control, mRNA can be isolated from these cultures and hybridized on microarray slides. The slides are analyzed and the genes that are modified by oxidative stress can be identified.

In this video, researchers make use of a robotic system to prepare a library of genome-wide yeast mutants, which are used to evaluate gene function. Due to the insertion specifically-engineered sequences, called genetic barcodes, into genes genomic DNA from mutant strains can be extracted from 4,000 to 6,000 individuals simultaneously and subjected to microarray analysis or sequencing. Based on the relative abundance of the barcode sequences, the fitness, of each mutant can be determined under multiple experimental conditions.

You've just watched JoVE's video on isolating nucleic acids from yeast. You should now understand the basic aspects of purifying nucleic acids such how to prepare yeast cells for lysis and how to perform different extraction and isolation procedures. As always, thanks for watching!