Yeast galactose induction

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Abstract

Protocol for induction of protein production using the GAL1 promoter in yeast.

Citation: Snehadri Sinha Yeast galactose induction. **protocols.io**
dx.doi.org/10.17504/protocols.io.gztbx6n
Published: 17 Jan 2017

Protocol

Step 1.

Prepare an overnight preculture by inoculating a single colony into 2-5 mL of approriate medium. Incubate overnight at 30 C with shaking.

If the protein to be produced is encoded in a plasmid, use knockout mix as selective medium (minimal media supplemented with amino acids, except for the one you have as an auxotrophic selection marker on your plasmid). Supplement sugar to the media if it doesn't contain it already; 2 % glucose.

Step 2.

The next morning, measure OD of your preculture; dilute as necessary to get an accurate measurement and calculate back to get the original OD.

Step 3.

Refresh the cell culture; spin cells down (around 3000 rpm, 5 minutes). Remove supernatant. Resuspend cells in selective media containing 0.5 % glucose; based on the ODs calculated, add media so that an OD of 0.2-0.5 will be acheived.

Step 4.

Grow for 4-6 hours at 30 C with shaking. Optimally, an OD of 1 will be acheived.

Step 5.

To induce the cultures, add 40 % galactose solution to the culture to acheive a final galactose concentration of 2 % (or alternatively, you can vary between 0.4-4 % as needed)

Step 6.

Grow in 30 C with shaking for 20 hours, then harvest cultures.