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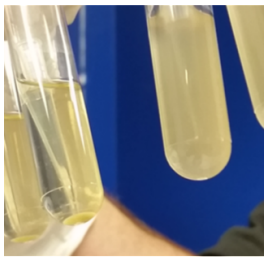
Yeast growth profile analysis



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Abstract

This method outlines a procedure for analyzing the growth curve of Baker's yeast, *Saccharomyces cerevisiae*, for the assessment of growth medias, the addition of metabolites to media, and the incorporation of heterologous genes to our yeast strain.

Troubleshooting

PROCEDURE

Day 1:
1. Streak a YPD-agar plate from a glycerol stock.
2. Incubate for 48-72 hours at 25-30 °C or until colonies are large enough to pick (1-2 mm diam.)

Day 2:
3. Check plates. There likely won't be any colonies that are large enough to pick

Day 3:
4. Colonies should be large enough to pick (1-2 mm diameter)
5. Propagate colony (ies) in 5 mL YPD media
6. Allow colonies to grow overnight at 30 °C shaking at 240-250 rpm

Day 4:
7. Propagate triplicates in 5 mL fresh YPD using 50 µL of the overnight growth added directly to the 5 mL of fresh media
8. Begin collecting data for the growth curve. This marks the t=0 time point.
9. To begin collecting time points, 50 µL of culture can be added to 750 µL sterile media (or ddH2O) using a polystyrene semi-microcuvette. Measure the absorbance at 600 nm
NOTE: Yeast grow more slowly than bacteria and time points can safely be collected every 2 hours. Additionally, after the fresh media is inoculated and time point 0 is collected, it can take up to 4 hours for any appreciable absorbance to be reached.
10. Time points data can be collected up to and including 48 hours. After 12 hours data was collected every 4 hours up to 24 hours and every 12 hours thereafter.
11. After an OD of 1.0-1.2 is achieved, yeast can be gently harvested at RT and fresh YPG added to induce protein expression
- Fix the cap of the culture tube so it is sealed (2nd stop)
- Using the Sartorius benchtop centrifuge (or other), spin at 500 x g for 3-5 minutes or until the supernatant is clear
- Gently remove the supernatant with a micropipette (save if interested in metabolite studies at - 20 °C in sterile 15-mL
conical tubes)
- Replace with 5 mL of fresh YPG media (warmed to 30 °C) and place back in the incubator at 30 °C. Continue collecting
time point data
12. Pellets can be retained and saved at the end of the study for additional analysis

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