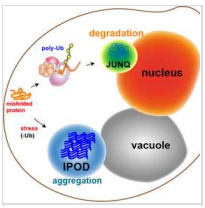

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Growth Curve Stress Test (Instructor Protocol)

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1 *Works for me* Share

This protocol is published without a DOI.

Yeast ORFans CURE

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ABSTRACT

This is the instructor protocol for

Growth Curve Stress Test
by Brian Teague,
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PREVIEW RUN

PROTOCOL CITATION

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MATERIALS TEXT

Equipment

- Shaking incubator (or preferably a roller drum in an incubator)
- Spectrophotmeter for measuring OD600

Materials

Yeast Nitrogen Base (YNB) **Sunrise**

- **Science Catalog #1500-100** Step 1

CSM-Leu-Ura Powder **Sunrise**

- **Science Catalog #1038-010** Step 1

Ammonium sulfate **Sigma**

- **Aldrich Catalog #A4418** Step 1

Uracil Yeast Culture Grade **Sunrise**

- **Science Catalog #1906-010** Step 1

L-Leucine Yeast Culture Grade **Sunrise**

- **Science Catalog #1980-010** Step 1

α-D-Glucose **Sigma**

- **Aldrich Catalog #158968** Step 3
- Test tubes for growing yeast cultures

SAFETY WARNINGS

Yeast and yeast media are not hazardous.

The yeast we are using are genetically modified. Make sure to inactivate them (by bleach or autoclaving, for example) before disposing of them, following your institution's guidelines.

Prepare 2x synthetic media 30m

1 Put **200 mL** of deionized water in a 250 ml bottle. Then add:

Yeast Nitrogen Base (YNB) **Sunrise**

- **0.855 g** **Science Catalog #1500-100**

CSM-Leu-Ura Powder **Sunrise**

- **0.335 g** **Science Catalog #1038-010**

Ammonium sulfate **Sigma**

- **2.5 g** **Aldrich Catalog #A4418**
- **5 mL** 100X uracil solution (**2 mg/mL**
  Uracil Yeast Culture Grade **Sunrise**
  Science Catalog #1906-010)
- **5 mL** 100X leucine solution (**12 mg/mL**
  L-Leucine Yeast Culture Grade **Sunrise**
  Science Catalog #1980-010)

30m

2 Autoclave **121 °C** for **00:30:00**

3 Cool, then add **50 mL** **40 Mass / % volume** gluco
α-D-Glucose **Sigma**
Aldrich Catalog #158968 using aseptic technique.

Prepare the yeast cultures

4 The afternoon or evening before lab, prepare a test tube with 5 ml 2xSDC media for each yeast strain you will be testing.

5 Pick a colony from each strain's plate and resuspend it in the media.

6 Grow **Overnight** at **30 °C** , either on a roller drum (preferred) or on an orbital shaker at **180 rpm** .

7 The next morning, several hours before class, measure the OD600 of each culture.

8 Prepare another test tube with 5 ml of 2xSDC media for each yeast strain. Subculture the overnights to an OD600 of 0.1. Place the tubes back in the incubator or shaker.

9 Optimally, grow the cultures for at least 3 hours before lab.

> The experiments will be more reproducible if the cells always start the experiment in logarithmic growth phase!

Instructor Tips & Common Student Errors

10 **Instructor Tips**

- This assumes that, once you or your students have identified yeast colonies with successful knockouts, you have struck them out on plates
- Log-phase growth is the most reproducible growth mode for yeast. This is why the experiment should start in log phase and why doubling time should be computed in log phase.
- Growing the cells in double-strength media means that they can be diluted 1:1 and still be in "complete" media for the stress experiment.
- Yeast cells are heavy and settle rapidly toward the bottom of a test-tube. Make sure you vortex them before measuring OD600; vortex again before subculturing; and vortex again before putting them in an assay plate.
- Computing doubling time is not optional! Because we're looking at an exponential phenomenon, a small difference in starting concentration is rapidly amplified and can "look" on a graph like a strain is growing slower.
- I thought everyone graduated highschool with a basic ability to use a spreadsheet? I am often surprised by the number of students that have trouble with "make a graph of this data."

11 **Common student errors**

- Not computing doubling time to compare growth, but just drawing conclusions from eyeballing a graph.
- Not vortexing the yeast cells before putting them in the assay plate.
- General data manipulation and interpretation difficulty