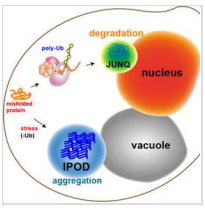

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Growth Curve Stress Test

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Yeast ORFans CURE

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ABSTRACT

This is one (fairly quantitative and reproducible) way to run a yeast growth study to analyze the effect of a stressor on yeast. It is not the only way! It is also less of a "protocol" and more of a "template" -- it measures the growth of a single yeast strain at one concentration of "chemical X." Makes sure to adapt it to your own needs!

PROTOCOL CITATION

Brian Teague 2022. Growth Curve Stress Test. **protocols.io** https://protocols.io/view/growth-curve-stress-test-cffjtjkn

KEYWORDS

yeast, phenotype, growth curve

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68811

PARENT PROTOCOLS

In steps of

Growth Curve Stress Test (Instructor Protocol)

GUIDELINES

Remember, if you see a difference between the knockout and the wild-type under stress, it could be that the stress is slowing down the knockout strain. However, there's another possible explanation: it could just be that the knockout strain is growing more slowly under all conditions. Hence the no-stress controls.

MATERIALS TEXT

Equipment

- Vortexer
- Microplate reader (with incubation and shaking)
- Sterile workspace (such as a biosafety cabinet or laminar flow hood)

Materials

- Corning COSTAR 96-well clear flat-bottom assay plate
- Wild-type yeast in double-strength synthetic complete media, OD600 ~ 0.1-0.2
- Knockout yeast in double-strength synthetic complete media, OD600 ~ 0.1-0.2
- A solution of Chemical X in water

SAFETY WARNINGS

Yeast and yeast media are not hazardous.

However, you may be using a hazardous "chemical X." Make sure to use appropriate PPE and engineering controls and dispose of it appropriately.

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

Assay setup

1

If possible, perform steps 1 and 2 in a sterile environment such as a biosafety cabinet or laminar flow hood.

Vortex the yeast culture briefly to resuspend the yeast cells.

2 1. Set up four wells according to the following table:

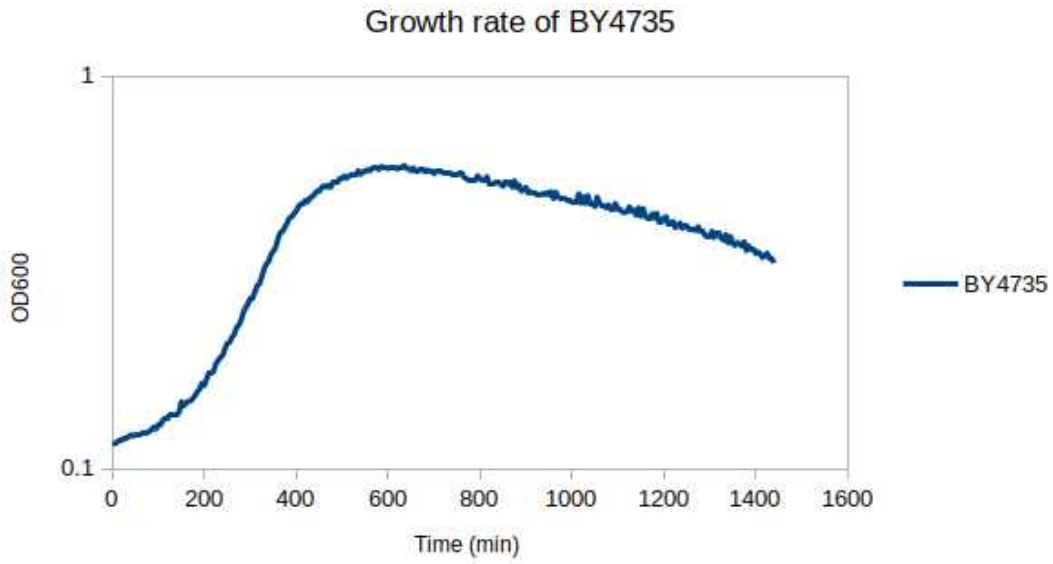
A	B	C	D	E
	Well 1	Well 2	Well 3	Well 4
Yeast culture (wild-type)	50 µl	50 µl	–	–
Yeast culture (knockout)	–	–	50 ul	50 ul
Chemical X, 100 mM	10 µl	–	10 µl	–
Water	40 µl	50 µl	40 µl	50 µl

3 Set up the plate reader as follows:
- Temperature: 30°C
- Mode: Kinetic
- Wavelength: 600 nm
- Interval: 5 minutes
- Total run time: 24 hours
- Shake before read: 30 seconds

4 Transfer the assay plate to the reader and read for 24 hours.

Data analysis

5 Plot each growth curve with the Y axis scale set to "logarithmic."



6 Find the part of the curve that is linear.

In this case, I might choose from 250 minutes to 350 minutes.

7 Find the OD600 reading at these two times.

At 250 minutes, my OD600 is 0.2086; at 350 minutes, my OD600 is 0.3583.

8 Compute the doubling time using a bit of math:

$$T_d = (t_2 - t_1) \cdot \frac{\ln(2)}{\ln(\frac{q_2}{q_1})}.$$

Where t_1 and t_2 are the start and end times you found in step 2, and q_1 and q_2 are the OD600 at those times.

Thus, for my BY4735 strain growing in YPD, the doubling time is 128 minutes.