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title: "Measuring Translation in Yeast using a PEST-nanoluciferase reporter"
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author: "Jared Bard"

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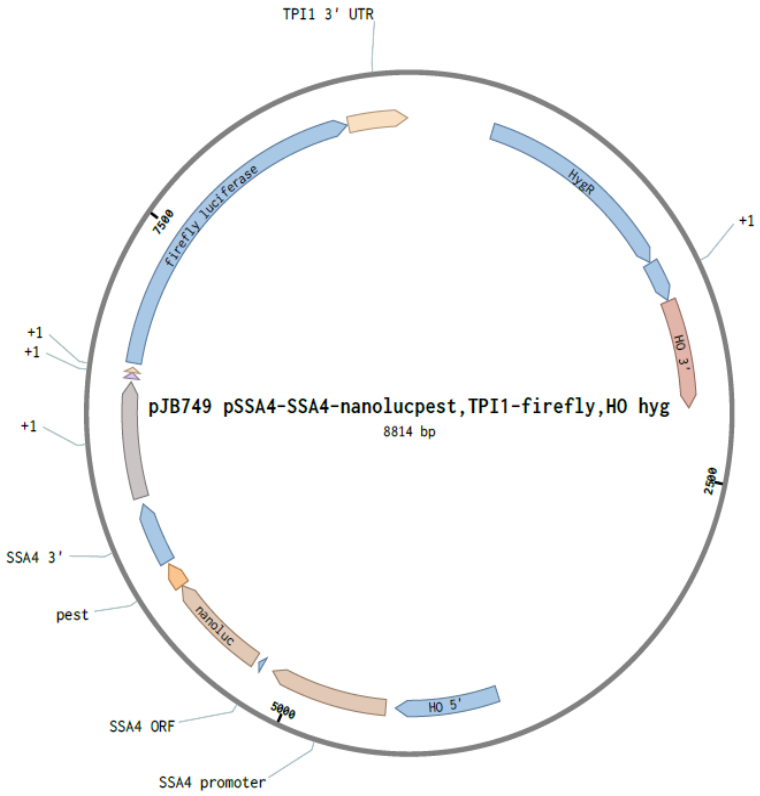
This protocol uses a nanoluciferase-PEST reporter to measure translation rate in the budding yeast *S. cerevisiae*.
The reporter design is based on that of Masser et al. Yeast 2016 May; 33(5): 191-200 (https://dx.doi.org/10.1002%2Fyea.3155).



Protocol

Reagents

Yeast Strain

- BY4742 + pJB749 (integrated at HO locus, selected with hygromycin)

Wet reagents

- yeast growth media (SC or YP plus sugar if required)
- Promega Nano-Glo (PRN1130)

Consumables

- 96-well 2mL deep well plate (Dot cat. no 229575)
- 96-well u-bottom microtiter plate (Thermo 1424571)
- 96-well PCR plate (Thermo AB-0600)
- 8-channel pipette (100uL or 200uL)
- 8-channel pipette (20uL) (Rainin P20 LTS)
- Breathe-easy sealing film (Sigma Z380059-1PAK)
- 384-well white plates (Corning CLS3574-50EA)

Instruments

- Multimodal microplate reader (Tecan Spark 20M)
- 96-well thermocycler
- 30 °C shaking incubator

Procedure

1. Grow cells
    - 3 days prior
        - streak strain from glycerol stock onto YPD plate, incubate at 30 °C
    - 2 days prior
        - pick one colony and grow in liquid YPD overnight with shaking at 30 °C
 culture can be stored at 4 °C
    - 1 day prior
        - assuming a doubling time of 85 minutes and a lag phase of 170 minutes, dilute yeast into 8 mL of fresh YPD such that it will grow to an $OD_{600} < 0.2$ the next day
        - grow at 30 °C with shaking
    - Day of morning
        - dilute to $OD_{600} < 0.05$ (such that experiment will start at $OD_{600} = 0.1$)

2. Prepare to measure translation
    - Calculate amount of Nano-Glo reagent required (will need 10 µL per measurement)
    - Prepare 0.5x Nano-Glo reagent by first mixing lysis buffer with 1:50 reagent, then diluting 1:1 with H_2O\ *diluting 0.5x is not necessary, but saves money and seems to work fine*
        - aliquot 10 µL reagent into 96-well PCR plate for later measurements
    - Calibrate the OD readings: Need to measure blank OD_{600} and calculate the pathlength of the measurement
        - Use multichannel to transfer 3x blank media and 3x yeast growth with known OD to U-bottom 96-well microplate
        - Read using JB_210712_A600_whole.mth on sparkcontrol (not magellan)
        - method first shakes for 5 seconds (linear, amplitude=4)
        - then reads every well at 600 nm with flashes=30 and settle time = 500 ms
 the settle time is important to get accurate readings
        - Using beer-lambert law ($A = \varepsilon lc$), calculate the pathlength (I got around 0.5 cm) and note the mean blank reading

3. Treat cells
    - Treatment will vary by experiment. In my experiments, I have been measuring the translation of two reporters during while depleting various translation factors, both before and after heatshock.
    - Transfer 0.25 mL yeast to 2 mL deep well plate
        - To account for variability in the luminescence readings, I always normalize my readings to a control strain. I also try to mix the order of my samples and do measurements in triplicate.
        - For instance, given reporter A and reporter B, and varying concentrations of drug I would arrange my columns like so

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	1	2	3	4	5	6	7	8
A	A	B	A	B	A	B	A	B
B	B	A	B	A	B	A	B	A
C	A	B	A	B	A	B	A	B

	1	2	3	4	5	6	7	8
A	-	-	+10	+10	+20	+20	+40	+40
B	-	-	+10	+10	+20	+20	+40	+40
C	-	-	+10	+10	+20	+20	+40	+40

- Cover 2mL deep well block in breath-easy film, tape to shaking platform and shake at 30 °C

4. Measure OD and luminescence (eventually want to calculate lum_per_OD to normalize for varying cell counts between samples)

    - Using multi-channel, transfer 150 µL to 96-well U bottom microplate, read OD_{600} as above
    - transfer 10 µL of culture to 10 µL Nano-Glo reagent in PCR plates (make sure to pipette up and down before transferring to mix any cells that have settled, then mix again 3x after transferring)
    - Transfer 15 µL to 384-well white plate *recommended to leave a well of blank between every well to prevent bleedthrough. Plate can be filled in after a few days*
    - Spin for 30 seconds in plate centrifuge
    - Incubate for 4 minutes before reading
    - Measure with e.g. JB_210712_Lum_A1toB12.mth (loop of 5 measurements, integration time = 250 ms, take average of measurements during analysis)

5. Heat shock and measure luminescence again

    - transfer 20 µL of culture to 96-well PCR plate
    - Treat at 42 °C using thermocycler
    - Re-read luminescence as above; I re-use the OD reading from above for normalization (assumes that during treatment the number of cells has changed a small amount)