Heat-Induction of GFP Release by TlpA-controlled Protein E Expression

Abstract

TlpA is a constitutively expressed protein, which binds to promotor P_{TlpA} and blocks the start of transcription of genes downstream of P_{TlpA}. [1][2] The binding to the promotor is reversibly deactivated by temperatures above 37 °C with full deactivation at 45 °C. Parameters influencing the final expression of the genes downstream of P_{TlpA} are the constitutive promotor and RBS of TlpA itself and the RBS of the P_{TlpA} downstream genes.

Protein E is a protein produced by phage Phi X 147, which (in nature) lyses the host cell after production of phage particles. [3] The exact mechanism of action of protein E has long been controversial and different models were proposed to explain its lytic function. It has been suggested that protein E activates a component of the E. coli autolytic system, that it inhibits cell wall synthesis in a manner similar to penicillin or that it oligomerizes to form a transmembrane tunnel, all leading to release of cytoplasmic content and ultimately cell death. [4][5][6]

However, it is now generally accepted that the most probable cellular target of protein E is an enzyme called Translocase I, encoded by the mraY gene. Translocase I plays an important role for cell wall biosynthesis and its inhibition leads to cell lysis.

In this experiment, gfp is expressed constitutively and accumulates inside the cells. Protein E will be induced with the TlpA thermosensitive repressor system at 45 °C for 3 h and the control group will be non-induced 37 °C. GFP release is measured with with a plate reader over the time of the induction. GFP can be replaced by another protein of interest, if the leakiness of the induction system and the cell lysis caused by protein E are sufficient for the final application.

Materials

- Luria Broth (LB) growth medium
- Plasmid 1 containing constitutively expressed TlpA and an antibiotic resistance gene i.e. piG17-2-002
- Plasmid 2 containing P_{TlpA} controlled protein E, a constitutively expressed GFP and another antibiotic resistance gene i.e. piG17-1-011
- Round bottom culture tubes 12 mL with breathable cap
- Antibiotics (according to the resistance genes, i.e. Kanamycin and Chloramphenicol)
- Flat bottom 96-well plate for plate reader measurement
- 1.5 mL Eppendorf microcentrifuge tubes

Equipment

- Two incubators with shaking function
- plate reader

Procedure

Day 1

1 Pick colonies of double transformants (biological triplicates, positive control, negative control) from plates or use cryo-stocks to inoculate 5 mL LB (with the appropriate antibiotics) in round-bottom tubes.

2 Incubate the cultures at 30 °C, shaking 250 rpm for 16 h (overnight).

Day 2

3 Inoculate 5 mL LB medium containing the appropriate antibiotics with the overnight cultures to reach an OD of 0.1 and incubate at 30 °C, shaking 250 rpm.
4 3.5 h after start of the incubation (step 3), prepare a 96 well transparent, flat-bottom measurement plate by adding 180 μL of PBS to each well and place it with a lid in the plate reader (30 °C) to warm up.
5 4 h after start of the incubation (step 3), divide the 5 mL cultures into 2 x 2 mL in fresh culture tubes.
6 Incubate the liquid cultures at **37 °C** and at **45 °C** for **3 h** shaking 250 rpm to induce the protein E production in the higher temperature condition.
7 Take 100 μL of the samples into an 1-5 mL Eppendorf tube every hour, starting at the same time as the induction. Place the round-bottom culture tubes back in the incubator.
8 Add 20 μL of each sample into 180 μL PBS in a well of the 96-well plate and measure OD_{600} and GFP fluorescence at a temperature of 30 °C. This is the total fluorescence of the sample. Add also 20 μL of the used LB medium to some wells to get blank measurements.
9 Spin down the remaining 80 μL of the sample in a tabletop microcentrifuge at 11'000 g for 5 min.
10 Repeat step 7 with the supernatant. This is the supernatant fluorescence.

Data analysis:

11 Use the formula $\mathrm{Fl}_{relative} = \frac{Fl(supernatant) - Fl(blank)}{Fl(total) - Fl(blank)}$ to find the ratio of released fluorescence to total fluorescence.
12 Plot $\mathrm{Fl}_{relative}$ of all samples for all time points.
13 Average the biological triplicates if desired, calculate standard deviation. Plot 37 °C vs. 45 °C induced samples.

References

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