Plate Reader experiment

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Abstract

This protocol covers the inoculation, cultivation, IPTG-induction and plate reader measurement of *E. coli* BL21 strains carrying constructs with various toehold switch devices from Green *et al*., 2014.

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Guidelines

The class will divide into four groups; each group will investigate one separate RNA device from Green *et al*.

Each device consists of two plasmids - a switch plasmid containing GFPmut3b, cis-repressed by a synthetic regulatory 5'UTR (untranslated region), denoted by H-xx-GFP, and a trigger plasmid containing a small regulatory RNA, denoted by T-xx, which binds to the switch sequence and switches on translation of GFP.

The following table shows the strains used by each group:

	Toehold switch device	Switch only control	non-specific trigger control
Group 1	T56, H56-GFP	H56-GFP	T117, H56-GFP
Group 2	T69, H69-GFP	H69-GFP	T56, H69-GFP
Group 3	T96, H96-GFP	H96-GFP	T69, H96-GFP
Group 4	T117, H117-GFP	H117-GFP	T96, H117-GFP

Before start

Materials/ reagents	# per group
Culture tubes	3x
Erlenmeyer flasks, 100mL	3x
12-well plate	1
Cuvettes	as needed
LB, sterile	as needed
H_2O, sterile	as needed

Stock solutions	Stock concentration
IPTG	100 mM
Kanamycin	100 mg/mL
Ampicillin	25 mg/mL

Protocol

Day 1: Inoculation

Step 1.

Inoculate 5 mL preculture of each strain using appropriate antibiotics.

H-xx switch plasmids: Kanamycin (final concentration 25 µg/mL)

T-xx trigger plasmids: Ampicillin (final concentration 100 µg/mL)

Incubate in a shaking incubator over night at 37˚C.

Day 1: Dilution

Step 2.

Early morning:

From each preculture, prepare a main culture in Erlenmeyer flasks.

Dilute the preculture 1:100 in a final volume of 20 mL LB (again using appropriate antibiotics).

Grow at 37˚C to an OD_{600} of 0.4-0.6 (start measuring OD_{600} after ≈ 1.5 hours).

Optional: If some cultures grow faster than others, flasks can be kept on ice to stop growth.

NOTES
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While measuring the ODs, the flasks should only be taken out for sampling and immediately returned to the incubator, otherwise growth will slow down!

Day 1: Induction

Step 3.

Morning:

Each culture should be measured in **duplicates,** growing one sample with H_20 (-) and one with IPTG (+) - resulting in 12 samples per group.

Induction experiments are performed in 12-well plates.

In a final volume of 2 mL per well, supplement with IPTG (final concentration 100 µM) or sterile H_2O.

Incubate plates in a shaking incubator for three hours.

LUNCH BREAK

Step 4.

LUNCH BREAK

Day 1: Plate Reader Measurement

Step 5.

While cells are incubating, prepare a pipetting scheme for your plate reader measurements. Two groups will share one 96-well plate.

Each culture should be pipetted in at least three technical replicates.

Pipet **75 µL LB** and **75 µL culture** per well.

While one person measures the cultures at the plate reader, the rest of the group should start RNA preparation (label tubes, cool centrifuge, prepare fume hood).

After pooling the duplicates, use **1 mL** of *E. coli* culture for RNA extraction.