

Killswitch Response Time Determination

Project: Sequencing

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Aim

To observe the time it takes the killswitch construct to arrest cell growth.

Principle

The killswitch is triggered when the genetic construct is exposed to an aerobic environment which causes a build up of CcdB toxin concentration inside the cell. This buildup eventually reaches a critical concentration after which the bacteria succumbs. Through computational modelling, we concluded that the arrest of growth can be observed around 50-70 minutes after the kill switch has been activated. We propose to check this experimentally. Furthermore, evidence from this experiment can be used to determine if we need to further finetune the K_d value and change the response time.

Materials Required

- LB media
- Cloning Kit
- Petriplates
- Conical flasks
- L-shaped glass rod
- Micropipettes and microtips

Procedure

- Clone the killswitch construct into E.coli K-12 using pET28-a backbone as directed by the [Cloning](#) protocol, and plates are prepared (Kanamycin)
- 100 ml of combined culture is prepared using LB media as directed by the [LB Medium Preparation](#) protocol in the 250 ml flask.
- Inoculate the culture with cloned bacteria and incubate at 37 °C in an anaerobic environment for 12 hours.
- Take 5 µl of culture and plate it using spread plate method.
- Expose the plate to aerobic environment for varying amounts of time as listed in Table1.
- Incubate the plates in anaerobic environment for 12 hours.
- Record the amount of colonies present on each plate.

	A	B	C
1	Sample	Time Exposed (min)	Amount of culture (μ l)
2	Ctrl 1	0	5
3	Ctrl 2	240	5
4	1	10	5
5	2	20	5
6	3	30	5
7	4	40	5
8	5	50	5
9	6	60	5
10	7	70	5
11	8	80	5
12	9	90	5
13	10	100	5
14	Total		60