

pH Dependency of FNR Promoter

Project: Sequencing

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Aim

To find the effect of different pH values on the FNR promoter.

Principle

As there are pH fluctuations in the process of harvesting and milling of sugarcane, it is important to make sure the killswitch is robust and functional over the probable range.

Materials Required

- LB media
- Cloning Kit (company)
- Various Tris buffers
- Different flasks
- Petriplates
- Eppendorf tubes

Procedure

- Genetic construct devised with FNR promoter followed by mCherry ([BBa_K2448004](#)).
- Cloned 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 prepared using LB media as directed by the [LB Medium Preparation](#) protocol in 250 ml flasks.
- Solutions are prepared according to the following table:

	A	B	C
1	Sample	pH	Amount of culture (ml)
2	Ctrl	7.0	5
3	1	5.0	5
4	2	5.5	5
5	3	6.0	5
6	4	6.5	5
7	5	7.5	5
8	6	8.0	5
9	7	8.5	5
10	8	9.0	5
11	9	9.5	5
12	10	10	5
13	11	10.5	5
14	Total		60

- The solutions are incubated in an anaerobic environment.
- Fluorescence is recorded for each of the samples and compared with the control.