FNR Characterisation

Project: Sequencing
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Created at: 2020-10-22T05:40:55.992335+00:00

THURSDAY, 22/10/2020

Aim

To characterise the functioning of theFNR promoter in the presence and absence of a hypoxic environment.

Principle

When the FNR promoter on enters a hypoxic environment, the formation of $[4Fe-4S]2+$ dimers is stimulated within the FNR-producing bacteria. These dimers are the transcription activators of the FNR promoter. They are able to bind to the promoter, thus allowing it to become active. Hence, mCherry downstream of a FNR promoter would only be expressed in a hypoxic environment.

Materials Required

- LB media
- Cloning Kit (company)
- Conical Flasks
- Petriplates

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)
- 300 ml of combined culture prepared using LB media as directed by the LB Medium Preparation protocol in 250 ml flasks (100 ml each).
- 100 ml of the culture labelled "Ctrl" is inoculated with bacteria cloned with control plasmid and incubated a 37 °C for 10 hours.
- The media is split into two flasks labelled A and B, inoculated with cloned bacteria and incubated at 37 °C for 10 hours keeping flask A in an anaerobic environment and flask B in aerobic environment.
- After incubation, the fluorescence is recorded.

Hypothesis

The ratio of the intensity of culture labelled A to the culture labelled Ctrl should be more than the ratio of intensity of culture labelled B to culture labelled Ctrl.