Caffeine Assay

Introduction

In order to test the overall system, a caffeine assay was performed to test that the nanobody is present and induction occurs. The assay also serves as a way to test leakage.

Materials

- Stock solutions of different caffeine concentrations
- LB-medium
- Antibiotic suitable for your construct
- Erlenmeyer flask
- Shaker in 37°C
- Spectrophotometer
- 1.5mL eppendorf tubes
- Plate reader

Procedure

1. Take your overnight culture and transfer 500uL into an Erlenmeyer flask containing 49.5mL of LB-medium and 50uL of the antibiotic suitable for your construct.
2. Place the Erlenmeyer flask on a shaker and incubate at 37°C for approximately 1.5h.
3. Measure the OD regularly with a spectrophotometer and let the cells reach an OD_{700} of 0.24 for your culture.
4. After incubation, directly aliquot of the culture 490uL into eppendorf tubes marked with corresponding caffeine solutions that will be added.
5. Pipette 10uL of the previously prepared caffeine dilutions into its corresponding eppendorf tubes with the culture.
6. Place the tubes on a shaker and incubate at 37°C overnight.
7. Measure fluorescence using a plate reader.