Plate reader assay – Landmine detection module

Materials

- Chemical solution of DNT or DNB [1g/L] dissolved in acetonitrile in 1mL a ampule
- Black (opaque) 96 well-plate, transparent bottom
- Plate reader machine
- Tris Buffer 200mM, pH 7 sterile solution
- Sterile distilled water
- O/N cultures of *Escherichia coli* carrying the LD constructs

Protocol

1. Bring down the DNT or DNB concentration to 300mg/L with Tris Buffer
2. Make the desired set of dilutions of DNT/DNB using either Tris buffer or distilled water (water seems to work better)
    a. Positive control: constitutively expressed mKate2
    b. Negative control: Working *E.coli* strain carrying no plasmid
    c. Number of replicates: 4 per condition (construct + DNT concentration)
3. Take cells from an O/N culture and bring the OD down to 0.8
4. Mix a 1:1 volume of cells and solution containing DNT/DNB at the desired concentration and pipet the mixture into the 96 well-plate. Final OD: 0.4
5. Measure Fluorescence at the plate reader. Using mKate2, the parameters must be close to the Excitation and Emission maximum of the protein (588 and 633nm respectively).
    a. Measuring time: up to 15 hours
    b. Time span between measurements: approximately 5 minutes (depending on the amount of samples)

Note

If LD4 and/or LD5 are used, Rhamnose induction must take place several hours before the plate reader experiments (at least 5 hours, but the curli experiments seem to point out that O/N induction is a better idea)