DYS SEE

in vivo Protein Expression Protocol.

iGEM Thessaly

Protocols

in vivo Protein Expression Protocol

This protocol is used as a general guideline to express a desired protein in protein expression *E. coli* cells (e.g. BL21 DE3, M15) and visualize them with a microplate reader. The following protocol is written for constitutive protein expression. For inducible expression (e.g. through IPTG), addition of the signal molecule is needed in each dilution step to ensure high expression.

1. Grow desired cells overnight in 5ml LB (~16h) at a shaken incubator, 37 degrees C / 210rpm
2. The following morning, measure the OD_{600} of overnight cultures
3. Dilute all cultures to OD_{600}= 0.05 in desired medium (LB, M9 etc)
4. Grow cells 37 degrees C /210 RPM until OD_{600}=0.4-0.6 (~2h)
5. Dilute all cells to the same OD_{600} (e.g. 0.4)
6. Load 160-200 ul of culture in a 96-well plate (do triplicates)
7. Measure the fluorescence (eGFP) or enzymatic activity (beta-lactamase, LacZ) of the expressed protein with a microplate reader.

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