

A1 A2 A7 in LB TEST 01/07/09

Description: Fluorescence estimation in green (GFP) and red (RFP)

Purpose: estimation of fluorescence in cultures of J100, J101 and J118 promoters expressing GFP or RFP

Methods: A flat-bottom non sterile plate is used. 96 wells are filled with:

- 200 ul LB+Amp,
- 200 ul J100 (RFP, non standard plasmid)
- 200 ul J101 (RFP, non standard plasmid)
- 200 ul J118 (RFP, non standard plasmid)
- 200 ul A1 (J100-GFP)
- 200 ul A2 (J101-GFP)
- 200 ul A7 (J118-GFP)
- 200 ul BBa_E0240 (negative control)

Dilution: cultures were incubated in 5ml LB+Amp 37°C 220 rpm overnight and then diluted 1:1000 (3 steps of 1:10 dilution, 500ul in 4.5ml)

After dilution, falcons were incubated 37°C 220 rpm for about 4 hours. LB is infected in 96-wells plate by dipping pipette in infected culture and then in LB.

Protocol:

- The plate is filled as described in Methods
- The instrument temperature was set at 37°C
- Dynamic cycle:
 - Shaking 15 s linear 3mm, waiting 10s and absorbance, GFP and RFP measurement.
 - Fluorescence gain setting: 50 to avoid overflow

