

A8 A9 induction test 02/07/09

Description: Induction test: we induce A8 and A9 with aTc

Purpose: estimation of induction curve using different concentration of aTc

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

- 200 ul LB+Amp,
- A8 induced with aTc 100ng/ml
- A8 induced with aTc 75ng/ml
- A8 induced with aTc 50ng/ml
- A8 induced with aTc 25ng/ml
- A8 induced with aTc 0ng/ml
- A9 induced with aTc 100ng/ml
- A9 induced with aTc 75ng/ml
- A9 induced with aTc 50ng/ml
- A9 induced with aTc 25ng/ml
- BBa_E0240 (negative control)
- A1 (J100-GFP) (positive control)
- 200ul A9 induced with aTc 75ng/ml in falcon and incubated with other dipping coltures

Dilution: coltures were incubated in 5ml LB+Amp 37°C 220 rpm overnight and then diluted 1:1000 (2 steps of 1:100 dilution (50ul in 500mlLB), 500ul in 4.5ml(500ul in 4,5ml LB))

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, excluding one well filled with 200ul A9 induced in falcon.

aTc dilution: Clontech aTc: 200ul of 2mg/ml.

Work concentration:

- aTc 1000x: aTc 100ug/ml in 50%ethanol -> 20ul aTc + 380ul 50% ethanol

our concentrations (2ul of dilution added to 200ul of coltures):

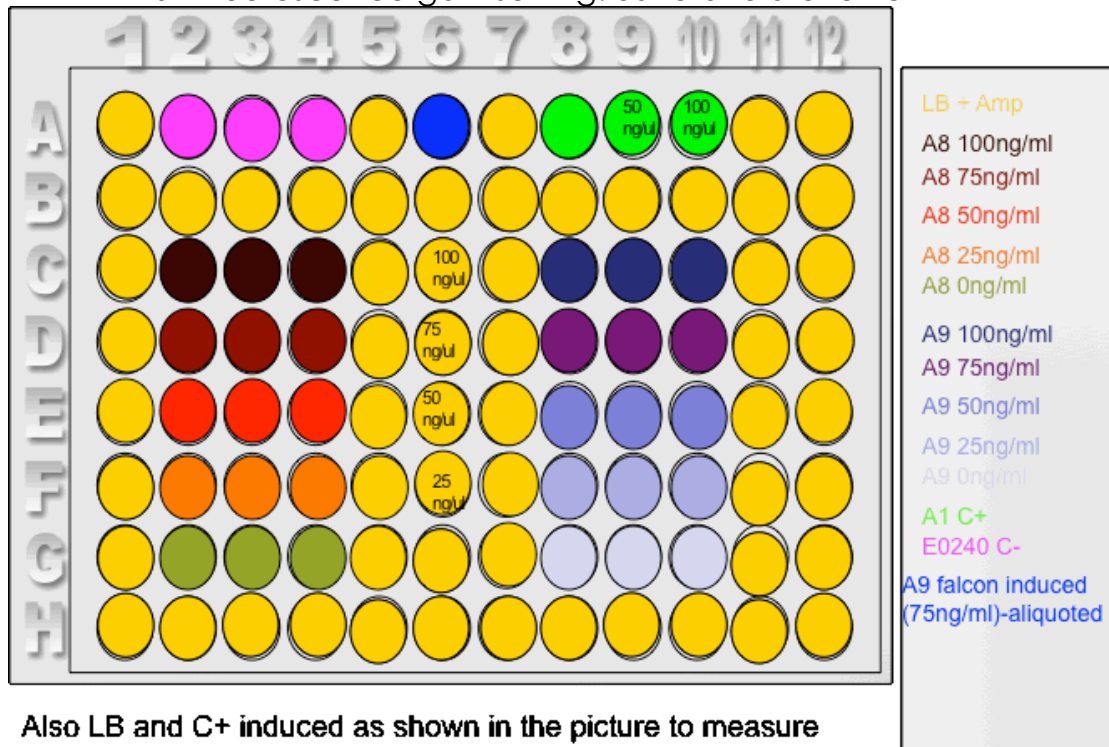
- 0ng/ml -> 0ng/200ul
- 25 ng/ml -> 0.025ng/ul->in 200ul 5ng -> 5ng/2ul =2.5ng/ul
- 50ng/ml-> 0.05ng/ml-> in 200ul 10ng -> 10ng/2ul=5ng/ul
- 75ng/ml -> 0.075 ng/ml -> in 200ul 15ng -> 15ng/2ul=7.5ng/ul
- 100ng/ml -> 0.1ng/ul -> in 200ul 20ng -> 20ng/2ul=10ng/ul

aTc dilutions:

- 0ng/ul
- 2.5ng/ul -> 1:2 dilution of 5ng/ul (10ul+10ulH2O)
- 5ng/ul-> 1:2 dilution of 10ng/ul (15ul+15ulH2O)
- 7.5ng/ul -> 3:4 dilution of 10ng/ul (15ul+5ulH2O)
- 10ng/ul -> 1:10 dilution of 100ug/ml=(100ng/ul) -> 5ul 100ug/ml in 45ul H2O

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 and GFP measurement.
 - Duration: 24hours, kinetic cycle: 20min
 - Fluorescence gain setting: 50 to avoid overflow



Also LB and C+ induced as shown in the picture to measure blank