

IPTG A14 A17 induction test TEST 29/09/09

Description: Induction test of A17 and A14 with different concentrations of IPTG

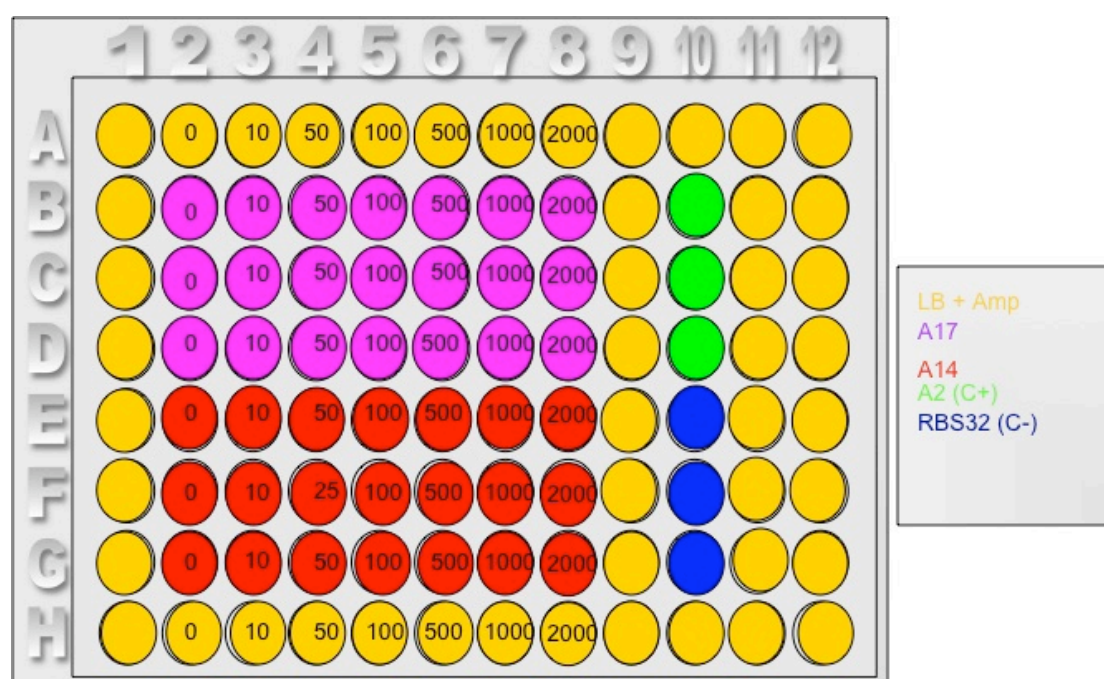
Purpose: Build the induction curve of A17 and A14 in LB medium. Positive control: A2 , negative control: RBS32.

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

- 200 ul LB+Amp,
- 200 ul bacterial cultures incubated overnight in LB+Amp 37°C 220 rpm and then diluted 1:100 in LB+Amp and incubated for further 4 hours.

We want to start from a normalized OD of 0.03 and arrive to 0.17 . So a measure has been performed to learn how to dilute cultures to start from the desired OD, using phormula:

Quantity to keep=(OD wanted/OD measured)*Desired final volume.



The induction with IPTG is in uM

Protocol:

Sampling: 5minutes after shaking and wait as always

Dilutions:

IPTG 200mM

2mM: 2ul 200mM in 200ul coltura
 1mM: 2ul 100mM in 200ul coltura
 500uM: 2ul 50mM in 200ul coltura
 100uM: 2ul 10mM in 200ul coltura
 50uM: 2ul 5mM in 200ul coltura
 10uM: 2ul 1mM in 20ul coltura
 0uM: 20ul ddH2O

200mM: 40ul
 100mM: 20ul 200mM + 20ul H2O
 50mM: 15ul 10mM + 15ul H2O
 10mM: 8ul 50mM + 32ul H2O
 5mM: 12ul 10mM + 12ul H2O
 1mM: 4ul 5mM in 16ul H2O