OD MEASUREMENTS

An OD measurement can be used to determine the time your bacteria need to reach the exponential phase. We used it in order to establish the viability of bacteria in different media.

MATERIALS:

- Cells
- LB medium with the appropriate antibiotic
- 1X PBS buffer
    - (additional buffers with different pH values)
- 10X MOPS buffer (at pH 5.5 and 6.5)
- 10X HEPES buffer (at pH 8.5)

PROCEDURE (IN A MEDIUM CONTAINING ONLY LB AND CHLORAMPHENICOL):

□ Incubate cells either over night or for at least 3h in 3-10ml LB+ chloramphenicol (25ug/ml chloramphenicol)

□ Measure initial OD

□ Centrifuge cells at 2000rpm, at 24°C for 3 min in order to obtain pellet. Discard the supernatant

□ Wash the pellet with 1 ml 1X PBS buffer

□ Centrifuge cells at 2000rpm, at 24°C for 3 min in order to obtain pellet

□ Discard PBS buffer

□ Resuspend cells in 1-5ml LB medium

□ Dilute the cells in LB-Chloramphenicol according to the OD measured at step 2.) to reach an initial OD of 0.1-0.5.. An initial OD of 0.1 was taken for our experiments.

Each measurement takes 1ml, thus the volume of LB depends on the number of measurements. Make sure your dilution volume is large enough for all the measurements you want to make.

□ Incubate the cells at 37°C and 200-250rpm and measure the OD each hour.

PROCEDURE (MEDIA WITH DIFFERENT PH VALUES):

□ Incubate cells either over night or for at least 3h in 3-10ml LB+ chloramphenicol (25ug/ml chloramphenicol)

□ Measure initial OD

□ Centrifuge cells at 2000rpm, at 24°C for 3 min in order to obtain pellet

□ Discard the supernatant

□ Wash the pellet with 1 ml 1X PBS buffer

□ Centrifuge cells at 2000rpm, at 24°C for 3 min in order to obtain pellet

□ Discard PBS buffer

□ Resuspend cells in 1-5ml LB medium

□ Prepare media with different pH values, each with a 1:1 ratio of buffer and LB+Chloramphenicol:

10X MOPS + HCl, adjusted to a pH of 5.5

10X MOPS, at pH 6.5

Water (as a control)

10X HEPES, at pH 8.5

□ Dilute the cells in the buffered media according to the OD measured at step 2.) to reach an initial OD of 0.1-0.5. An initial OD of 0.1 was taken for our experiments

Each measurement takes 1ml, thus the volume of LB depends on the number of measurements. Make sure your dilution volume is large enough for all the measurements you want to make.

□ incubate the cells at 37°C and 200-250rpm and measure the OD each hour.