Growth Curve

1. **Preparation of LB media under different conditions:**

   1) Under different pH value:

   prepare 7 kinds of LB media

   (Adjust pH value with 5M NaOH and 5M HCl):

   48ml LB media x3 at pH4,

   48ml LB media x3 at pH5,

   48ml LB media x3 at pH6,

   48ml LB media x3 at pH7,

   48ml LB media x3 at pH8,

   48ml LB media x3 at pH9,

   48ml LB media x3 at pH10

   2) Under different temperature:

   prepare the general LB media :

   48ml x3 for 27°C, 48ml x3 for 32°C, 48ml x3 for 37°C

   3) Under different salinity:

   prepare 5 kinds of LB media:

   48ml LB media x3 for 0.17M NaCl,

   48ml LB media+0.22g NaCl x3 for 0.25M NaCl

   48ml LB media+0.92g Nacl x3 for 0.50M NaCl

   48ml LB media+1.62g NaCl x3 for 0.75M NaCl

   48ml LB media+2.32g NaCl x3 for 1.00M NaCl

2. Mix *Bacillus subtilis* culture with prepared LB media:

Extract 1μl *Bacillus subtilis* culture glycerol stocks to 3ml LB media and incubate for 12hours. After incubation, extract 2ml culture to each flask containing 48ml prepared LB media.

3. Collect the data

All the experiments with every condition are operated in biosafety cabinet with triplicate.

1) Under different pH value:

Put all the flasks in 37°C incubators, 260 rpm, for 14 hours. Extract 200μl cell culture to 96 well plate and measure the OD level once an hour.

2) Under different temperature:

Put 3 flasks in 27°C incubator,3 flasks in 32°C incubator, and 3 flasks in 37°C incubator, 260rpm, for 14 hours. Extract 200μl cell culture to 96 well plate and measure the OD level once an hour.

3) Under different salinity:

Put all the flasks in 37°C incubator with 260 rpm for 14 hours. Extract 200μl cell culture to 96 well plate and measure the OD level once an hour.