

## Creating a growth curve of the culture

A growth curve is monitored to find out the log phase of the growth of *E. coli*. The characterization of competition between promoters is done in the log phase growth of *E. coli*.

1. An empty vector *E. coli* colony is cultured in LB containing antibiotics overnight to become saturated
2. 5ml LB with no antibiotics are prepared
3. Every 0.5 hours, 100uL of the saturated culture is transferred into clean LB tubes  
3 technical replicates are taken, i.e. 3 tubes for every time point.
4. The falcon tubes are put into shaker incubator at 37°C
5. At the 6th hour, all tubes are taken out from the incubator. 100uL of culture is transferred from each tube to a well of a 96-well plate
6. The OD600 values of the wells are measured by a plate reader
7. OD value is plotted against time to show at which OD the log phase starts  
Log phase starts at OD=3 in our experiment

## Preparing starter cell cultures

1. Colonies with plasmids containing 1 promoter, 2 promoters and 3 promoters, 3 colonies each are picked into a falcon tube containing LB with antibiotics
2. Incubate the falcon tubes at 37°C in the shaker incubator overnight
3. Serially dilute the saturated solution to find out at which dilution ration the solution does the OD value equals to that when the log phase starts.
4. Dilute all experimental sets to that concentration

## Taking culture sample

1. Diluted cultures are put into shaker incubator at 37°C
2. Every 0.5 hours, the cultures are taken out of the incubator and 3 biological replicates and 2 technical replicates of 100uL are taken from each tube and put into a 96-well plate
3. The plate is chilled at 4°C and falcon tubes are put back into incubator immediately after transferring
4. After a 7.5 hours span of taking samples every 0.5 hours, the 96-well plate is taken to the plate reader for fluorescence and OD values measurement
  - a. LB without antibiotics is used as blank.
  - b. Fluorescence value is taken at an excitation wavelength of 586nm and an emission wavelength of 611nm.
5. Fluorescence/OD is plotted against time to show the trend and comparison between different colonies.