Growth Curve Measurement

Materials

Strains and Culture Media

Wild-type *E. coli* BL21(DE3)
Engineered *E. coli* strains
LB broth
Sterile double-distilled water (ddH_2O)

Consumables

Sterile 96-well microplates
Sterile culture tubes
Sterile pipette tips

Equipment

Shaking incubator
Spectrophotometer
Micropipettes
Microbial growth curve analyzer (MGC-200, Ningbo Scientz Biotechnology Co., Ltd., China)

Procedure

Preparation of Bacterial Cultures

1. Prepare three independent bacterial cultures for each wild-type and engineered strain as biological replicates.
2. Measure the OD_{600} of each culture using a spectrophotometer.
3. Dilute each culture separately with fresh LB medium to an initial OD_{600} of 0.01, preparing a final volume of 2 mL for each biological replicate.

Preparation of the 96-Well Plate

1. Transfer 200 μL of each diluted bacterial culture into six separate wells of a sterile 96-well microplate, generating six technical replicates for each biological replicate.
2. Arrange the three biological replicates of each strain in separate rows, with six technical replicates per row.
3. Fill the peripheral wells of the microplate with 200 μL of sterile ddH_2O to minimize evaporation and reduce edge effects during incubation.
4. Add sterile, uninoculated LB medium to designated blank wells, using the same volume as the bacterial samples, to measure the background absorbance of the culture medium.

Growth Curve Measurement

1. Place the prepared 96-well plate into a microplate reader.
2. Set the incubation temperature to 37 °C and enable orbital shaking at 200 rpm.
3. Measure the optical density after a short time 1000 rpm at 600 nm (OD_{600}) at 5 minutes intervals for a total of 20 hours.
4. Record the OD_{600}values of all sample wells and LB blank wells at each time point.

Data Processing

1. Subtract the corresponding LB blank OD_{600} value from each bacterial sample measurement.
2. Calculate the mean OD_{600} of the six technical replicates at each time point to obtain one growth curve for each biological replicate.
3. Calculate the mean and standard deviation (SD) across the three independent biological replicates for each strain.
4. Plot the blank-corrected OD_{600} values against cultivation time to generate growth curves, presenting the results as mean ± SD (n = 3 biological replicates).
5. Compare the growth profiles of engineered strains with those of the wild-type control to evaluate potential effects of tRNA pool engineering on bacterial growth.