

1. Add 50ml LB culture medium and 50ul chloramphenicol into a 250ml conical flask, pick Adda solid puncture bacteria with toothpicks and enrich them in the liquid culture medium (t-37 °C, 150rpm, and the culture time is about 10-12 h).
2. The enriched Adda bacteria solution was dipped in and coated or underlined on LB AGAR solid medium with the corresponding concentration of chloramphenicol (chloramphenicol volume: medium volume = 1:1000), and the culture was conducted for about 20h to isolate a single colony.
3. Three single colonies of similar size were selected and placed in three 50ml LB medium (250ml conical flask) with chloramphenicol added. The culture time was about 10-12 h at t-37 °C with a rotation speed of 150rpm.
4. Mark Numbers 1 through 9 on the nine 50 ml LB medium.
Add 50 µl chloramphenicol and 75µM aTc(1.8µl)in 1 to 3;
Add 50 µl chloramphenicol and 75µM aTc(1.8µl) as well as 50µM 2—AP(67.5µl) in 4 to 6;
Add 50 µl chloramphenicol and 75µM aTc(1.8µl) as well as 150µM 2—AP(200µl) in 7 to 9;
Take 500µl the 3 bottles of single colony liquid come from the previous step,then add it respectively in 1 to 3 and 4 to 6 as well as 7 to 9.T-37°C, 150rpm to cultivate.
5. Measurement: fluorescence (excitation light at 485nm, absorption light at 512nm) and OD600 were measured by ELIASA at 2h, 4h, 6h, 8h, 10h and 12h after induction respectively.Each bottle absorbed 1ml of bacterial liquid in a 1.5ml EP tube, centrifuged at RCF =13910g for 2min, poured the culture medium, added 1ml 1×PBS and mixed by suction.200ul was added to each hole of the 96-well enzyme plate, 3 holes were added to each EP tube,and the blank was PBS(The most peripheral circle of the 96-hole plate is not used) , and data were recorded.