# 2025 NEFU_China

## Genome-Wide Mutation

ABSTRACT

This protocol is designed to transform three plasmids (MP6-K plasmid, pLB1s-PBAD-tnaC-mcherry-cmr plasmid, and pYB1a-P23119-tnaE$^{S40F}$DCBA plasmid) into BWΔtrpRΔtnaAB competent cells. Subsequently, genome-wide mutation is achieved through induced culture in M9 glycerol-Y medium, laying a foundation for improving tryptophan synthesis levels.

BEFORE STARTING

1. Extract the three target plasmids (MP6-K, pLB1s-PBAD-tnaC-mcherry-cmr, and pYB1a-P23119-tnaE$^{S40F}$DCBA) respectively, ensuring that the plasmid purity and concentration meet the requirements of subsequent transformation.

2. Co-transform the three plasmids into BWΔtrpRΔtnaAB competent cells by chemical transformation.

8. Inoculate the picked single colonies into LB liquid medium containing Amp, Kana, and Smr respectively, and incubate with shaking at 37°C and 200 rpm for 12 hours to obtain seed bacterial solution.

3. Prepare M9-Y medium (the components and their dosages are as follows: 2 ml of 5×M9 solution, 1 ml of 20% glycerol, 20 μl of magnesium sulfate ($MgSO_4$), 1 μl of calcium chloride ($CaCl_2$), 10 μl each of ampicillin (Amp), streptomycin (Smr), and kanamycin (Kana), 50 μl of 100 g/L yeast solution, 100 μl of 12-arabinose (12-Ara), 4 μl of chloramphenicol (Cmr, screening concentration: 12 mg/L), and 100 μl of seed bacterial solution; make up to 10 ml with double-distilled water (DDW)). Transfer the prepared M9-Y medium into a sterile culture tube and incubate with shaking at 30°C.

4. Measure the OD value of the bacterial solution using a spectrophotometer to determine whether the bacteria enter the logarithmic growth phase.

5. When the bacteria are in the logarithmic growth phase (or before reaching the stationary phase), take 100 μl of the bacterial solution and transfer it to the newly prepared M9-Y medium, and repeat the above culture and detection steps.

6. Continue the subculture for 10 rounds.