2024 NEFU_China

1. Take pLB1s-ndmDtB as template (template plus 20~50ng/50μL), use high-fidelity enzyme, concatenate primers for PCR (25 cycles, reduce unnecessary mutations), amplify 3~4 tubes.
2. Purify the PCR product and do not recover the gel.
3. Take 200~300ng for DpnI digestion.
4. Transfer to BW/TPR sensory state, A+S resistance, incubate at 37℃ for 12~16h, try to cultivate until the colony is bigger.
5. Prepare a deep well plate, add 600μL of induced transformation medium (LB+0.5 mM theophylline +A100+S50+0.2 % arabinose+1 mM IPTG) into each well in three shots.
6. Pick the monoclones into each well (select large and full colonies as much as possible) Note: choose four wells to pick the unmutated TPR+DtB (prepared in advance) as the control experiment, the other 92 wells to pick the TPR+DtBs.
7. Cultivate in 96-well plate shaker at 30℃ and 850rpm for 24h.
8. Take 200 μL in ultra-clean and put it into 96-well plate, and detect it under the enzyme labeling instrument.
9. Record the group with stronger fluorescence than the control group, take 50 μL of bacteria, and 300 μL of sample for HPLC detection (400 μL of bacterial solution left in the deep-well plate in the previous experiment was used for this step).
10. Sequence the mutants with improved yield in the HPLC assay to identify their mutation sites. Note: This experiment is applicable to TPR-mCherry, which is a sensor with very slow colony fluorescence formation. If a sensor with fast and strong fluorescence is developed later, the first step is to perform a preliminary screening of the naked eye color to reduce the workload.

Note: This experiment is applicable to TPR-mCherry, which is a sensor with very slow colony fluorescence formation. If a sensor with fast and strong fluorescence is developed later, the first step is to perform a preliminary screening of the naked eye color to reduce the workload.