



شرکت سلول بافت زیست (سبز)

Bacteria Transformation (cloning)

Protocol

1. Prepare a culture of the bacteria in LB Broth medium to reach an OD₆₀₀ of about 0.5 (3-4 hours in shaker incubator)
2. Pellet the bacteria by centrifugation (7000 g, 3 min)
3. Resuspend in 1 ml cold CaCl₂
4. Incubate on ice for 20-30 min
5. Pellet the bacteria by centrifugation (7000 g, 3 min)
6. Resuspend in 0.7 ml cold CaCl₂
7. Incubate on ice for 20 min
8. Pellet the bacteria by centrifugation (7000 g, 3 min)
9. Resuspend in 0.3 ml cold CaCl₂
10. Divide the bacterial suspension in two parts of 150 µl volume each.
11. Add 20 µl of the plasmid (ligation product or any other plasmid) to one of the bacterial suspension tubes. (The other tube serves as negative control)
12. Incubate on ice for 30 min
13. Rapidly move to 42° C and incubate for 90 seconds.
14. Rapidly move on ice and incubate for 5 min.
15. Add 1 ml of fresh LB Broth medium without antibiotics and incubate for 1 h in 37° C

Bacteria Transformation (cloning) Protocol

16. Transfer 300 μ l of the suspension to a LB agar plate containing appropriate antibiotics (and IPTG 0.1 mM and Xgal 0.02-0.04 mg/ml if white/Blue screening). Centrifuge the rest of the suspension and resuspend it in 300 μ l and transfer to another similar plate. The negative control tube is transferred to one plate.
17. Incubate in 37° C for an overnight.
18. Incubate for an overnight and check the colonies by colony-PCR. (For Colony-PCR it is better to select primers that match to the vector backbone not the insert to minimize the risk of false positive results due to non-ligated free insert strands.
19. Extract plasmid from positive colonies by a miniprep plasmid extraction kit and check the plasmid by enzymatic digestion. (Note that for plasmid extraction the bacteria should be cultured in 5 ml LB Broth for 12-16 h in shaking incubator).