Schwer Lab

Transformation of home-made, chemically competent DH5α bacteria

1. Remove 200-μL aliquot of bacteria from lab stock -80°C freezer and directly place tube into wet ice. Let completely thaw.
2. Place required number of microcentrifuge on wet ice.
3. Flick the tube gently to resuspend thawed bacteria. Do not let warm up. Aliquot 100 μL bacteria into ice-cold microcentrifuge tubes on ice (use P200 tip).
4. Add DNA (~20-100 ng) to bacteria. Do not pipet up and down. Instead, gently swirl tip in tube. Return tube to ice.
5. Incubate 30 min on ice. Check water bath and adjust to 37°C if needed.
6. Heat shock bacteria for 5 min, 37°C.
7. Return tube to ice and incubate for 2 min.
8. Add 300 μL 2×-LB (no selection antibiotics!).
9. Tape microcentrifuge tube into microcentrifuge rack. Place rack on its side into 37°C-shaker. Shake 225-250 RPM, 45 min. [During this time, place agar plates into 37°C incubator].
10. Plate 50 μL and 150 μL per 2X LB/Carbenicillin plate (use glass beads for plating).
11. Incubate plates o/n at 37°C.