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Liquid Culture transformed Bacteria DH5α with pET.1a.C162

AIM: liquid culture of transformed bacteria for miniprep

Equipment

- Petri Dish with LB agar media + antibiotics CARB 50 µg/ml or CM
- LB broth sterilized by Bunsen burner
- Antibiotics: Carbenicillin 50mg/mL (CARB 50 mg/ml stored at -20°C) or Chloramphenicol (CM 25 mg/ml)
- Sterile Erlenmeyer or Falcon of 50 ml
- Inoculator = inoculation loop of 1µL
- Pipette p200 + associated cones (p200/20), Pipet p10 + paired cones
- Plastic graduated pipette (10ml or 20 ml)
- Electric Propipet

Transformed Bacteria
- DH5α pET43.1a.C162

1. In 50 ml sterile Falcon tubes (or Erlenmeyer previously autoclaved and sterilized by Bunsen Burner (use aluminium as lid to cover the Erlenmeyer)) we add 20 ml of LB broth and 20 µl of antibiotic: CARB (50 mg/ml)
2. Mix by pipetting up and down 6 times
3. Using an inoculation loop of 1 µL, touch a colony of transformed bacteria: DH5 pET43.1a.C162on the petri dish. Immerse and dip the inoculation loop in the liquid media and stir.
4. On a new petri dish LB/CARB spread the rest of the bacterial colony (zig-zag movement)
5. Place the liquid culture in the incubator at 37°C for 14 hrs at 150 rpm. Maintain the lids on top using tape but do not close the tubes.
6. After 7 hrs we observe a blurring of the solution, which proves the presence of bacteria in the media.
7. Place the petri dish in the incubator at 37°C for 14 hrs and then stored a 4°C.

After 14 hours:

8. In contained in Erlenmeyer the liquid cultures are transferred in falcon tubes of 15 or 50 ml
9. The tubes are centrifuged (don't forget to balance the machine and use the adaptor) at 5°C for 10 mins at 3 600 - 4 500 g
10. Discard the supernatant and the rest of media is removed using a pipette p1000 (beware not to pipette the pellet)
11. The Pellet is stored at -20°C & named: pET43.1aC162 col 1, col 2, col3, col 4