Isolate amplified plasmids from bacteria

Introduction

The plasmid extraction protocol is used to purify up to 30 µg of plasmid DNA (<12kb) from 1-4 ml overnight cultures of E. coli in LB (Luria-Bertani) medium. This technique allows plasmid DNA purified with this product to be immediately ready for use. Phenol extraction and ethanol precipitation are not required. High-quality plasmid DNA is eluted in a small volume of Tris Buffer or deionized water.

Reference:
1. TOOLS Plasmid Mini kit / (N.d.). https://www.toolsbiotech.com/product_detail1.php?id=547&cateId=334
2. Hsieh, Y. C., Chiang, M. C., Huang, Y. C., Yeh, T. H., Shih, H. Y., Liu, H. F., ... & Cheng, Y. C. (2018). Pparα deficiency inhibits the proliferation of neuronal and glial precursors in the zebrafish central nervous system. *Developmental Dynamics*, *247*(12), 1264-1275.

Materials

- cultured Bacteria strains
- Plasmid extraction kit (stored dry at room temperature (15-25°C) for up to 12 months)
  - RNase A (powder)
  - Buffer P1
  - Buffer P2
  - Buffer P3
  - Buffer PD
  - Buffer PW
  - Buffer EB
  - Spin Columns CP3
  - Collection Tubes（2 mL）

Procedure

Materials set-up

1. Prepare P1 buffer: Add 0.5 mL of Buffer P1 to the provided RNase A and mix well, then add the mixture (P1 buffer and RNase A) back into the residual P1 buffer. Mix everything well and store at 2-8°C.
2. Add 200 mL ethanol (96-100%) to buffer PW before use (check bottle label for volume).
3. Check buffers P1, P2, and P3 before use for salt precipitation. Dissolve the buffers every time there is any precipitate by warming to 37°C. Do not shake buffer P2 vigorously.
4. Close the bottle containing buffers P2 and P3 immediately after use to avoid acidification due to contact with CO2 in the air.

Operation

5. Harvest 4 mL of bacterial cells in a microcentrifuge tube by centrifugation at 12,000 rpm (approximately 13,400 × g) in a conventional table-top microcentrifuge for 1 min at room temperature (15-25°C), and remove all traces of supernatant until all medium has been drained.

6. Resuspend pelleted bacterial cells in 200 µl of buffer P1.

7. Add 200 µl of buffer P2 and mix thoroughly by inverting the tube 10 times.

   Note: Mix gently by inverting the tube. Do not vortex, as this will result in the shearing of genomic DNA.

8. If necessary, continue inverting the tube until the solution becomes viscous and slightly clear.

   Do not allow the lysis reaction to proceed for more than 5 min.

9. Add 300 µl of buffer P3 and mix immediately and thoroughly by inverting the tube 10 times. The solution should become cloudy.

   Note: To avoid localized precipitation, mix the solution thoroughly and immediately after the addition of buffer P3. Do not

10. vortex, as this will result in the shearing of genomic DNA.

11. Centrifuge for 10 min at 12,000 rpm (approximately 13,400 × g) in a table-top microcentrifuge. A compact white pellet will form.

12. Add the supernatants from step 5 to spin column CP3 (place the CP3 in a collection tube) by decanting or pipetting.

13. Centrifuge for 1 min at 12,000 rpm (approximately 13,400 × g). Discard the flow-through.

14. Wash spin column CP3 by adding 400 µl of buffer PD and centrifuging for 1 min at 12,000 rpm(approximately 13,400 × g). Discard the flow-through.

15. Wash spin column CP3 by adding 700 µl of buffer PW (ensure the ethanol [96-100%] has been added to buffer PW) and centrifuging for 1 min at 12,000 rpm (approximately 13,400 × g). Discard the flow-through.

16. Centrifuge for an additional 3 min at 12,000 rpm (approximately 13,400 × g) to remove residual wash buffer PW.

    Note: Residual wash buffer will not be completely removed unless the flow-through is discarded before this additional centrifugation. Open the CP3 lid and letting the samples stand at room temperature for a while.

17. Place spin column CP3 in a clean 1.5 mL microcentrifuge tube. To elute DNA, add 50-100 µl of buffer EB or water (pH 7.0-8.5) to the center of each spin column CP3, let the samples stand for 2-5 min, and then centrifuge for 2 min at 12,000rpm (approximately 13,400 × g).

18. Store the collected DNA solution –20°C.

    Note: The pH of the elution buffer may affect the efficiency of the extraction. Please ensure that the pH is within the range of 7.0-8.5 if using water as the elution buffer.