Plasmid DNA Extraction from *E. coli* Using the Alkaline Lysis Method

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[Abstract] It is a quick and efficient way to extract *E. coli* plasmid DNA without using commercial kits. This technique was invented by Birnboim and Doly (1979).

Materials and Reagents

1. RNAase (Life Technologies, Invitrogen™)
2. Isopropanol (EM Science)
3. Ethanol Absolute (200 Proof) (VWR Chemical)
4. Tryptone
5. Yeast extract
6. NaCl
7. Glucose
8. EDTA
9. 0.2 N NaOH
10. SDS
11. KOAc
12. Potassium acetate
13. Glacial acetic acid
14. Tris-HCl
15. Luria-Bertani broth (LB) medium: Bacto-tryptone (BD Biosciences), yeast extract (BD Biosciences) (see Recipes)
16. Resuspension solution (P1 buffer) (see Recipes)
17. Lysis solution (P2 buffer) (see Recipes)
18. Neutralizing solution (P3 buffer) (see Recipes)
19. TE (see Recipes)

Equipment

1. Table-top centrifuge

Note: Use the highest speed for all centrifugation steps in this protocol.

2. 1.5-ml Eppendorf tube
3. 37 °C heat blocker

Procedure

Note: All steps except of steps 9 and 10 are carried out at RT.

1. Grow bacterial (*E. coli*) culture in LB medium with appropriate antibiotics at 37 °C overnight (O/N) with shaking. For >10 copies plasmid, 3 ml cell culture is usually enough.
2. Transfer O/N culture to a 1.5-ml eppendorf tube, and spin down cell culture (twice) at highest speed for 1 min at table-top centrifuge.
3. Discard the supernatant. To remove the liquid completely by upside down tube onto a piece of paper towel for a few seconds.
4. Add 100 µl of resuspension solution (P1 buffer) into each tube, and vortex to completely resuspend cell pellet.
5. Add 100 µl of lysis solution (P2 buffer) and mix by gently inverting the tube 5-6 times. The solution should quickly turn transparent and become more viscous indicating bacterial lysis has taken place.
6. Add 150 µl of neutralizing solution (P3 buffer) and mix by inverting the tubes several times. At this point bacterial chromosomal DNA is usually seen as a white precipitate.
7. Centrifuge the tubes at highest speed for 10 min.
8. Carefully transfer the supernatant (try to not disturb the white precipitate) to a new labeled 1.5-ml eppendorf tube with a 1 ml pipette.
9. Add 2.5-3 volume of 200-proof cold ethanol (stores at -20 °C) to each tube and mix by inverting the tubes a few times.
10. Spin down plasmid DNA precipitate (transparency pellet) at highest speed for 10 min.
11. Discard the supernatant and remove the remaining liquid as much as possible by leaving the tube upside-down on a piece of paper towel, then keep the tubes in a tube holder and air dry for 10-20 min. To dry faster, keep tubes at 37 °C heat blocker. DNA precipitate turns white when dry.
12. Resuspend the DNA pellet with 50 µl TE. Completely dissolve the pellet by pipetting solution several times.

    Note: Large amounts of RNA is present in the DNA sample. Therefore, for subsequent reactions, for example, to digest plasmid DNA, add 1-5 µl (1 mg ml^{-1}) RNAase to the digestion solution to completely remove RNA. Or, add RNAase directly to the resuspension solution with a final concentration of 1 mg ml^{-1}.

Recipes

1. LB medium
   1% Tryptone
   0.5% yeast extract
   200 mM NaCl
2. Resuspension solution (P1 buffer)
   50 mM glucose
   10 mM EDTA
   25 mM Tris (pH 8.0)
   Store at 40 °C
3. Lysis solution (P2 buffer)
   0.2 N NaOH
   1% SDS
   Store at room temperature
4. Neutralizing solution (P3 buffer)
   3 M KOAc (pH 6.0)
   For 100 ml solution, 60 ml 5 M potassium acetate (49.07 g potassium acetate in 100 ml H_2O)
   11.5 ml glacial acetic acid and 28.5 ml H_2O, store at room temperature.
5. TE
   1 mM EDTA
   10 mM Tris-HCl (pH 8.0)
   Note: P1, P2, P3 buffers from the QIAGEN DNA extraction kit also work well.

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References

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