E. coli Genomic DNA Extraction

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[Abstract] This protocol uses phenol/chloroform method to purify genomic DNA without using commercial kits.

Materials and Reagents

1. Tris base (Calbiochem-Behring)
2. Proteinase K (Sigma-Aldrich)
3. Phenol\chloroform (1: 1) (EM Science)
4. 200 proof ethanol (Pharmco-AAPER)
5. RNAase (Life Technologies, Invitrogen™)
6. Ethanol
7. SDS
8. EDTA
9. Tryptone
10. Yeast extract
11. NaCl
12. LB medium (see Recipes)
13. TE buffer (see Recipes)
14. Lysis buffer (see Recipes)

Equipment

1. Tabletop centrifuge (Eppendorf)
2. 1.5 ml Eppendorf tube
3. Incubator
4. Gloves

Procedure

1. Transfer 1.5 ml of the overnight *E. coli* culture (grown in LB medium) to a 1.5 ml Eppendorf tube and centrifuge at max speed for 1min to pellet the cells.

2. Discard the supernatant.
   Note: Remove as much of the supernatant as you can without disturbing the cell pellet.
3. Resuspend the cell pellet in 600 µl lysis buffer and vortex to completely resuspend cell pellet.
4. Incubate 1 h at 37 °C.
5. Add an equal volume of phenol/chloroform and mix well by inverting the tube until the phases are completely mixed.
   Note: Do not vertex the tube—it can shear the DNA.
6. CAUTION: Phenol is a very strong acid that causes severe burns. Chloroform is a carcinogen. Wear gloves, goggles and lab coat, and keep tubes capped tightly. To be safe, work in the hood if possible.
7. Spin at max speed for 5 min at RT (all spins are performed at RT, unless indicated otherwise). There is a white layer (protein layer) in the aqueous: phenol/chloroform interface.
8. Carefully transfer the upper aqueous phase to a new tube by using 1 ml pipetman (to avoid sucking the interface, use 1 ml tip with wider mouth-cut 1 ml tip-mouth about ~2 mm shorter).
9. Steps 4-6 can be repeated until the white protein layer disappears.
10. To remove phenol, add an equal volume of chloroform to the aqueous layer. Again, mix well by inverting the tube.
11. Spin at max speed for 5 min.
12. Remove aqueous layer to new tube.
13. To precipitate the DNA, add 2.5 or 3 volume of cold 200 proof ethanol (store ethanol at -20 °C freezer) and mix gently (DNA precipitation can be visible).
    Note: DNA precipitation may simply diffuse, which is normal. Keep the tube at -20 degree for at least 30 min (the longer the better) and then spin it down (see Steps 15-16). You should see DNA pellet. It looks transparency when it is wet and turns to white when it becomes dry.
14. Incubate the tube at -20 °C for 30 min or more.
15. Spin at max speed for 15 min at 4 °C.
16. Discard the supernatant and rinse the DNA pellet with 1 ml 70% ethanol (stored at RT).
17. Spin at max speed for 2 min. Carefully discard the supernatant and air-dry the DNA pellet (tilt the tube a little bit on paper towel). To be faster, dry the tube at 37 °C incubator.
18. Resuspend DNA in TE buffer.
    Note: Large amounts of RNA will be present in the DNA sample. So, 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 lysis buffer with a final concentration of 1 mg ml^{-1}.

19. Check isolated Gemonic DNA on an agarose gel.
    Note: We expect to see bands with smear patterns from high to low MW range, although most of DNA fragments are accumulated at high MW on the gel. So, if you see most of DNA fragments are small, very likely your DNA got degraded.

Recipes

1. LB medium
   1% tryptone
   0.5% yeast extract
   200 mM NaCl
2. TE buffer
   10 mM Tris-Cl (pH 8.0)
   1 mM EDTA (pH 8.0)
3. Lysis buffer (10 ml)
   9.34 ml TE buffer
   600 ul of 10% SDS
   60 µl of proteinase K (20 mg ml^{-1})

Acknowledgments

This protocol was adapted from Andrew Binns' lab protocol collections at the University of Pennsylvania (USA).

References

1. Maniatis T., E.F. Fritsch, and J. Sambrook (1982). Molecular Cloning A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Springs Harbor, NY.

