

Practical write up for C8P:Molecular Biology( 2)  
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## Genomic DNA Isolation from *E.coli*

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### Materials and Reagents

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1. Tris base
2. Proteinase K
3. Phenol\chloroform (1: 1)
4. 200 proof ethanol
5. RNAase
6. Ethanol
7. SDS(Sodium dodecyl sulfate)
8. EDTA(Ethylenediaminetetraacetic acid)
9. Tryptone
10. Yeast extract
11. NaCl

12. LB medium (see Recipes)
13. TE buffer (see Recipes)
14. Lysis buffer (see Recipes)

**Recipes- 1.LB medium**-1% tryptone, 0.5% yeast extract, 200 mM NaCl 1%,Agar 1.5%

First,weigh and then add the required amount of Tryptone,yeast extract,NaCl(1gm,0.5gm amd 1gm respectively) and dissolve in 100ml double distilled water,mix well, add 1.5 gm of agar.(For 100ml of LB media).

**2. TE buffer**-10 mM Tris-Cl (pH 8.0), 1 mM EDTA (pH 8.0)

Make the followings first,

1M Tris 60.57 g in 500ml double distilled or mq water, pH adjusted to 8.0 using HCl.

1M EDTA 186.1g in 500m double distilled or mq water, pH adjusted to 8.0 using NaOH.

Now, to make 100ml of TE buffer, Take 1ml of 1M Tris solution, take 0.1ml of 1M EDTA solution, and make up the volume upto 100ml with mq water.(The volumes to be taken were calculated using  $V_1S_1=V_2S_2$  formula) Mix well and autoclave to sterilize.

**3. Lysis buffer** (10 ml) 9.34 ml TE buffer, 600 ul of 10% SDS, 60  $\mu$ l of proteinase K (20 mg ml<sup>-1</sup>)

## Equipment

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1. Tabletop centrifuge
2. 1.5 ml Eppendorf tube
3. Incubator

### Precedure-

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  $\mu$ l lysis buffer and vortex to completely resuspend cell pellet.

4. Incubate 1 h at 37  $^{\circ}$ 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 vortex 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  $^{\circ}$ 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 transparent when it is wet and turns to white when it becomes dry.*

14. Incubate the tube at -20  $^{\circ}$ 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 the RNA, add 1-5  $\mu$ l (1 mg ml<sup>-1</sup>) RNAase to the digestion solution to completely remove RNA. Or, add RNAase directly to lysis buffer with a final concentration of 1 mg ml<sup>-1</sup>.*

19. Check isolated Genomic 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.*