Aim 51 Isolation of Genomic DNA from Bacterial Cells

Introduction

In the presence of SDS bacterial cells are lysed with lysozyme and their treatment with chloroform-isoamyl alcohol mixture is helpful for removing of protein. Precipitation with alcohol gives DNA.

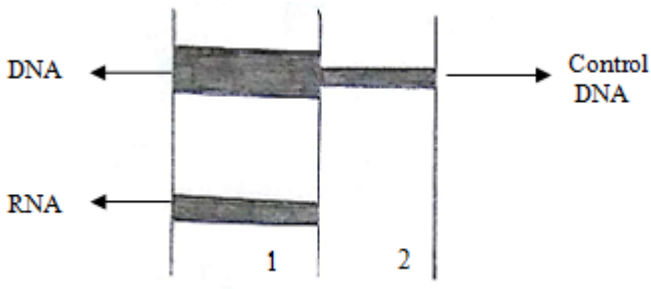
DNA preparations can be deproteinized by using Sodium perchlorate. SDS and associated proteins get removed by Sodium perchlorate at high concentrations. Sodium perchlorate also used to prevent precipitation of proteins with nucleic acid in ethanol precipitation reaction.

Requirments

1. Lysozyme solution – 10 mg/ml
2. *coli* cells
3. SDS solution - 25%
4. Extraction medium
5. Sodium perchlorate – 10 M
6. Alcohol – 95%
7. Saline sodium citrate (0.15 M NaCl with 0.015 M sodium citrate)
8. Chloroform isoamyl alcohol (24:1)
9. Centrifuge
10. Spectrophotometer

Procedure

1. *E.coli* cells are grown in Luria Bertani media.
2. The cells are centrifuged.
3. 5 g of wet packed bacterial cells are taken.
4. Suspend them in 50 ml of extraction medium.
5. 2 ml of lysozyme solution is added to above suspension.
6. Incubate at 37°C for 30 min.
7. 5 ml of SDS solution is added and mixed well.
8. The mixture is heated upto 60°C for 10 min.
9. Cool the above solution by keeping at room temperature.
10. Sodium perchlorate solution is added and mixed well.
11. Equal volume of chloroform: isoamyl alcohol is added to above solution.
12. Stir well for 30 min. and centrifuge at 10,000 g for 5 min.
13. There is formation of three layers. Upper layer is collected which contains nucleic acids.
14. 2 volumes of chilled 95 % ethanol are added to the collected upper layer.
15. The precipitate is centrifuged and dissolved in saline sodium citrate and stored at 4°C.
16. Note down the concentration of DNA in the sample by using spectrophotomeric method and test the purity of DNA sample.
17. Use 50 µL RNase (10 µg/µL) for the digestion of RNA for 10 min.


DNA
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