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Chapter 22

Isolation of bacterial chromosomal DNA: Principles, techniques, and analytical applications

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1. Introduction:

The isolation of bacterial DNA involves several crucial steps, including lysozyme breakdown of the cell wall, detergent lysis, disruption of protein-nucleic acid complexes, and phenol: chloroform extraction for protein removal (Doyle, and Doyle, 1990; Tripathi et al., 2018). Alternative techniques utilizing guanidine hydrochloride have been effective against species resistant to detergent lysis, such as mycobacteria. However, the extraction process can be influenced by cell physiological conditions, which may inhibit lysis or result in the production of substantial quantities of extracellular polysaccharides or glycocalyx material (Sambrook and Russell, 2001; Srivastava et al., 2022). Utilizing extremely young cultures is potentially effective, albeit requiring substantial quantities to obtain sufficient cells. The boiling technique, though expedient, yields DNA with minimal purity, suitable for PCR reactions (Wilson, and Walker, 2010; Tripathi et al., 2013a, b). Typically, these techniques start with liquid cultures but can be adapted for cells cultivated on solid media by resuspending them in an appropriate buffer. Cultures grown on solid media are more prone to encountering difficulties due to increased extracellular material production.

2. Materials Required:

1. Centrifuge machine
2. Hot plate
3. Incubator
4. Inoculating loop
5. 1.5 ml Eppendorf tubes
6. Sterilized pipettes and tips
7. Bacterial isolate grown on an agar petridish
8. Autoclaved water

3. Solution Preparation:

1. Phenol/chloroform: iso-amyl alcohol (1:1 volume ratio)
2. Mix 10% Hexadecyltrimethylammonium Bromide and 5M NaCl in equal parts.
3. Prepare RNAse solution by dissolving 100 mg of RNAse enzyme in 10 millilitre of sterile molecular grade distilled water.
4. Inactivate DNAse by incubating the solution at 90°C for 10 min and store at -20°C.
5. To make Proteinase K solution, mix 100 mg of the enzyme with 5 millilitres of sterile distilled water and refrigerate.
6. Mix up a pH 8.0 solution with 1 mM EDTA and 10 mM Tris-HCl.
7. Mix 10g of SDS with 100 millilitres of distilled water, then refrigerate.
8. With 5M NaCl, dissolve 292.2 grams of NaCl in a final volume of 1 litre.
9. Sterlize by autoclaving.
10. Use autoclaved water of the highest quality for molecular biology purposes.

4. Procedure:

1. In the first step, thoroughly dissolve a loop of colonies from a cultivated agar plate in 500 µl of TE.
2. Thoroughly mix in 30 µl of 10% SDS.
3. Add three microlitres of proteinase K (20 mg/ml), raise the temperature to 37°C, and leave for sixty minutes.
4. Thoroughly mix in 100 µl of 5M NaCl. Then add 80 µl of CTAB/NaCl, swirl the tube gently, and leave it for ten minutes at 65°C.
5. Pour in an equal volume of iso-amyl alcohol: phenol (25/24:1), well mix, and spin for five minutes at 8000 rpm. Spoon the upper layer into a fresh tube.
   Continue with step 5.
6. Add 50 µg/ml of RNAse and leave at 37°C for 30 minutes.
7. Carry out step five again.
8. To the DNA solution add 2 volumes of 100% cold ethanol and 1/10 volumes of 5M NaCl. Give it a night at -20°C or 60 minutes at -80°C.
9. After 20 minutes of 12,000 rpm spinning, remove the supernatant.
10. Wash in 70% ethanol, spin, and make sure everything dries completely.
11. Mix in 50–200 µl TE buffer and refrigerate.

5. Precautions:

1. Choose a suitable bacterial culture and ensure it is in the logarithmic growth phase.
2. Handle bacterial cells gently to avoid shearing of DNA molecules.
3. Use appropriate buffers and enzymes for cell lysis and DNA extraction, following manufacturer instructions.

4. Minimize exposure of samples to UV light or other sources of DNA-damaging radiation.
5. Maintain consistent and appropriate incubation temperatures during the extraction process.
6. Use RNase treatment to remove RNA and prevent contamination of the DNA sample.
7. Store extracted DNA in appropriate conditions (e.g., at -20°C) to maintain integrity and stability.

6. References:

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