Deep Science Publishing
https://doi.org/10.70593/978-93-49307-52-0_23

Chapter 23

Extraction and purification of plasmid DNA from bacteria: Techniques and applications in molecular biology

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1. Introduction:

Plasmids, self-replicating DNA molecules distinct from the bacterial chromosome, are typically circular, covalently closed, and super-coiled (Sambrook, and Russell, 2001; Srivastava et al., 2022). These molecules harbor genes encoding specific functions, like antibiotic resistance, which are advantageous but not vital for regular cellular activities. In recombinant DNA technology, plasmid DNA serves as vectors for transporting foreign DNA (Wilson and Walker, 2010). They replicate autonomously within host cells and exhibit discernible traits aiding in their identification. Genetic engineering leverages recombinant DNA technology to merge genes with plasmid vectors and replicate them in host cells. Through molecular biology techniques, scientists can synthesize and utilize a diverse range of genes and their products across various sectors including industry, medicine, and agriculture (Tripathi et al., 2013). A modified alkaline lysis method, based on Birnboim et al., 1979, is employed to extract plasmid DNA from *E. coli* efficiently. This protocol allows for rapid and cost-effective recovery of substantial quantities of plasmid, suitable for cloning or restriction analysis (Brown, 2010; Tripathi et al., 2018). However, the plasmid purity attained may not be sufficient for sequencing purposes.

Table 1: Buffers required

Buffer	Components	Storage
Buffer 1	50 mM Tris-HCl, pH 8.0; 10 mM EDTA; 100 mg/mL RNase A	Store at 4°C
Buffer 2	0.2 M NaOH; 1 % (w/v) SDS	-
Buffer P3	3 M Potassium acetate, pH 5.5	-

2. Procedure:

2.1. Inoculation:

1. Inoculate 5 mL of LB broth, supplemented with appropriate antibiotics, using a single colony.
2. Incubate overnight at 37°C.

2.2. Cell Harvest:

Centrifuge the bacterial culture at a maximum of 5000 rpm and room temperature for 1 minute.

2.3. Cell Lysis:

1. Completely remove the supernatant after centrifugation.
2. Resuspend the bacterial pellet in 350 µL of buffer 1.
3. Pipette out 350 µL of buffer 2 and gently mix by inverting the container (avoid using a vortex).
4. Incubate for a maximum of 5 minutes at room temperature. (Note: Prolonged incubation may result in fragmented genomic DNA.)
5. Add 400 µL of buffer 3, handle with care, and gently mix by inverting (avoid vigorous shaking!).
6. Centrifuge the sample for 10 minutes at room temperature and 6000 rpm.
7. With precision, transfer the supernatant to a new tube, ensuring minimal contamination from the pellet material.
8. If necessary, repeat centrifugation (Step 7) to fully eliminate the pellet.

2.4. Precipitation using Isopropanol:

1. Add 1 unit of isopropanol to the tube. Cool the mixture on ice for a minimum of 2 minutes. (Note: Incubation time can be extended to one hour if necessary.)
2. Centrifuge the sample for 5 minutes at room temperature and 6000 rpm.
3. Remove the supernatant without disturbing the DNA pellet.
4. Wash the pellet by adding 500 µL of 70% ethanol without resuspending it. Centrifuge the sample for 5 minutes at room temperature and 6000 rpm.
5. Place the pellet in a thermoblock and heat at 65°C for 10 minutes until dry.

2.5. Recovery of Plasmid DNA:

1. Resuspend the pellet in 50-100 µL of pure water or preferred buffer, such as TE buffer.
2. Optional: Incubate the sample at 65°C for efficient DNA dissolution.

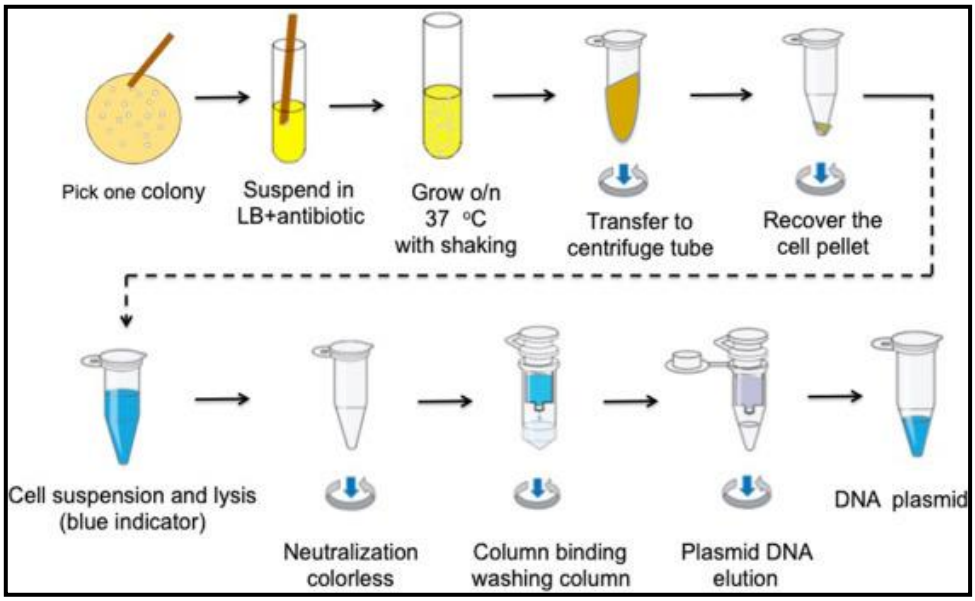

Figure 1: Plasmid isolation by Alkaline lysis

2.6. Preparation of Agarose Gel for Electrophoresis:

1. Prepare a 0.8% Agarose solution in 1X TBE.
2. Pour the agarose into gel casting trays with combs.
3. Once the gel solidifies, carefully remove the combs without damaging the wells.
4. Place the gel in a horizontal electrophoresis tank filled with 1X TBE buffer.
5. Proceed with electrophoresis by adding approximately 5 µL of plasmid DNA eluate and 2 µL of Sample buffer into each well, along with a DNA marker or ladder.
6. Conduct electrophoresis at a consistent voltage, monitoring the migration of DNA.
7. After electrophoresis, immerse the gel in Ethidium Bromide (EtBr) solution for 30 minutes to stain the DNA.
8. Handle the gel with gloves, rinse with water, and visualize DNA bands under UV light using a Gel Documentation system/transilluminator.
9. Determine DNA concentration based on the ladder utilized.

Figure: 2. Plasmid DNA along with molecular weight marker

3. Precautions:

1. Choose a bacterial culture known to harbor the plasmid of interest.
2. Use appropriate buffers and enzymes for cell lysis and plasmid DNA extraction, following manufacturer instructions.
3. Handle bacterial cells gently to avoid shearing of DNA molecules, especially the plasmid.
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 plasmid DNA sample.
7. Perform phenol-chloroform extraction and ethanol precipitation steps carefully to purify the plasmid DNA.
8. Verify the quality and quantity of isolated plasmid DNA using spectrophotometry or other suitable methods.
9. Store isolated plasmid DNA in appropriate conditions (e.g., at -20°C) to maintain integrity.

4. Reference:

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