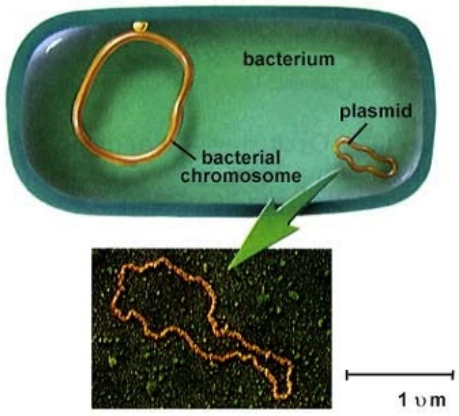
2. (A) PLASMID ISOLATION FROM *ESCHERICHIA COLI* USING ALKALINE LYSIS METHOD

&(B) VISUALIZATION AFTER ELECTROPHORESIS ON AN AGAROSE GEL

AIMA : TO ISOLATE THE PLASMID FROM TRANSFORMED BACTERIAL CELLS



PRINCIPLE

Plasmid is a double stranded, circular extra chromosomal DNA of bacterium. Plasmid DNA is much smaller in size than chromosomal DNA. Plasmid DNA exists mostly in a supercoiled form.It is used in recombinant DNA experiments to clone genes from other organisms and make large quantities of their DNA.

Purification of plasmid DNA from *Escherichia coli* using alkaline lysis is based on the differential denaturation of chromosomal and plasmid DNA in order to separate the two.

Bacteria are lysed with a solution containing sodium dodecyl sulfate (SDS),a strong anionic detergent and sodium hydroxide. SDS helps in the bacterial lysis. During this step, chromosomal as well as plasmid DNA are denatured owing to the alkaline conditions.

In the next step, neutralization with potassium acetate allows only the covalently closed plasmid DNA to reanneal and to stay solubilized. Most of the chromosomal DNA and proteins precipitate in a complex formed with potassium and SDS, which is removed by centrifugation.

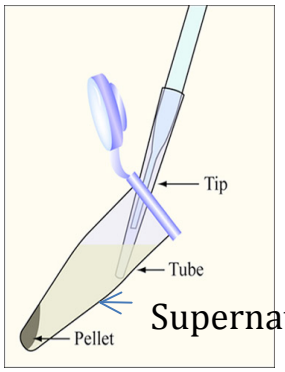
The plasmid DNA is concentrated from the supernatant by ethanol precipitation.

MATERIALS OR CHEMICALS REQUIRED: 2 ml culture of transformed bacterial cells, Eppendorf tubes, Micropipettes, Centrifuge, Solution-1, 2 & 3, TE Buffer, Isopropanol, 70% Ethanol, RNase.

Solution-2 should be prepared fresh. If you are using a week old solution, ensure that there is no precipate, and if there is a precipitate, warm it for a few minutes (42C water bath)

PROCEDURE:

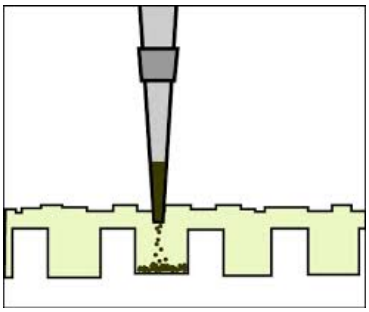
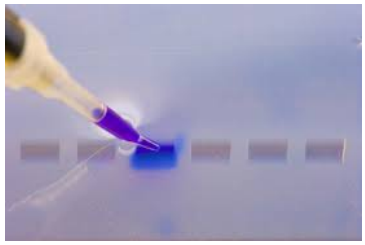
1. Keep solution-1 on ice, solution-2 at RT, and solution-3 on ice.
2. Take 2ml of bacterial culture in to a centrifuge tube (Use the 2ml tubes, not 1.5ml) , label it and balance it with an identical tube with an equal volume Water can be used for balancing) and centrifuge it for 2 min at 10,000 rpm at room temperature. Discard the spent medium (supernatant).



3. Add 200µl of **ice-cold**Solution-1 (this is a cell resuspension solution) to the bacterial pellet, followed by 2 microlitre of RNAse solution,
4. Vortex the bacterial pellet in the solution, so that bacterial formed pellet is completely resuspended. {SOLUTION 1: Glucose: Maintains osmolarity and helps in lysis of the cell; Tris-HCl: Used for maintaining the pH (acts as Buffer); EDTA: Inhibits nucleases by chelating the divalent metal ions (Ca^{2+} or Mg^{2+})}
5. Add 200µl of solution-2 (Cell lysis solution),invert the tube gently 3-4 times for mixing.**DO NOT VORTEX (to avoid bacterial genomic DNA contamination during plasmid isolation)** and leave it at room temperature for 5 min. (The solution should become clear) {SOLUTION 2: SDS (Sodium dodecyl sulphate): Make the plasma membrane porous by binding to lipids; NaOH: Create alkaline environment, helps in denaturation of genomic DNA and proteins}
6. Add 300µl of **ice cold** solution-3 (neutralization and plasmid renaturation solution) mix it by inverting, leave the tube on ice for 10 min. White precipitates is formed, and then centrifuge the tube for 10 min at 10,000 rpm at room temperature. {SOLUTION 3: Potassium acetate (CH_3COOK)-glacial acetic acid: Neutralizes alkaline pH, precipitates protein and forms SDS-Protein complexes, and helps in renaturation of plasmid DNA.
7. Take the supernatant carefully in a fresh 1.5 ml micro centrifuge tube (**don't take any white precipitate**).
8. Add 700µl of isopropanol (for DNA precipitation), mix by inverting and incubate for 2 min. Centrifuge the tube at 10,000 rpm for 10 min at room temperature and discard the supernatant.
9. Add 1ml of 70% ethanol; mix it by inverting the tube then centrifuge at 10,000 rpm for 5 min at room temperature. Discard the supernatant by decanting into the liquid waste. Invert the tube on a clean tissue paper.
10. Remove all traces of ethanol either by air drying or putting it in hot air oven with lid open.
11. Resuspend the DNA in 50µl of Tri-EDTA (TE) buffer. Vortex briefly (30 seconds). Allow the pellet to dissolve by incubating at room termperature for 15 minutes.

AIM B: VISUALIZATION OF PLASMID AFTER ELELCTROPHORESIS ON AN AGAROSE GEL

When plasmid DNA is isolated and run on an agarose gel, you may observe 2, 3 or even 4 or more bands. Hopefully the majority of your isolated DNA will be supercoiled DNA, but other forms can also crop up. How these forms will show up on an agarose gel (in terms of relative migration speeds) is shown in the diagram below The plasmid DNA is visible because the agarose gel and buffers contain ethidium bromide that intercalate in the DNA and show a purple fluorescence when exposed to UV light.

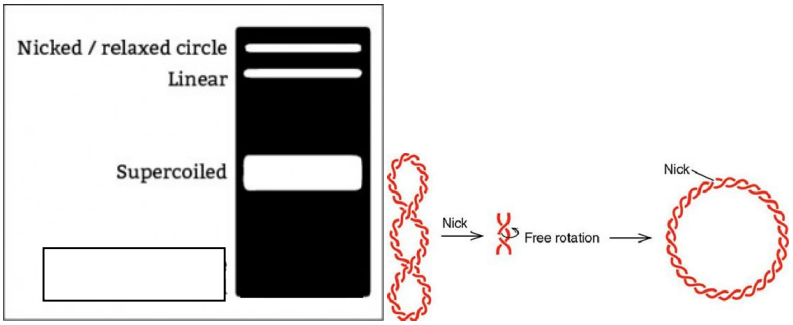




MATERIALS REQUIRED: Agarose gel (1% agarose, already prepared), pipettes, DNA loading dye (from lab, Composition:30% (v/v) glycerol0.25% (w/v) bromophenol blue,0.25% (w/v) xylene cyanol FF)

PROCEDURE:

Wear gloves: Ethidium bromide is toxic

1. Take 10 µl of the dissolved DNA pellet in a 0.5 ml centrifuge tube and add 2ul volume of 6x concentrated DNA loading dye and mix it. Store the remaining DNA on ice and then to -20C.
2. Place the agarose gel in the electrophoresis tank containing electropherisis buffer (Tris-Acetate-EDTA, TAE). As the buffer also contains ethidium bromide, and the agarose gel contains ethidium bromide, use gloves when handling the gel.
3. Load the above sample on the agarose gel (already prepared). In one lane load a mol weight marker DNA. When all the samples are loaded, close the lid, connect it to the power supply provided and left it to run for half an hour. (Red electrode is positive) (DNA will run towards the positive electrode)
4. See the gel in Gel Doc under UV light. Observe and record the plasmid DNA bands.



What bands do you see and why ? What does the size of the plasmid DNA appear to be ?