Specific protocols for alkaline lysis differ from laboratory to laboratory, however they are all based on the same principal. The first stage is to grow the selected bacterial colonies in a small volume (3-5ml) of LB broth containing the selection antibiotic. The bacteria are pelleted and resuspended in a resuspension buffer. This buffer is often a basic pH Tris buffer, which helps to denature DNA, and EDTA (ethylenediaminetetraacetic acid) that binds divalent cations destabilizing the membrane and inhibiting DNases (enzymes that degrade DNA). In addition, RNases are also added to degrade the released RNA.

Next, the bacteria are lysed with strong alkali (Sodium Hydroxide (NaOH)) and detergent (Sodium Dodecyl Sulfate (SDS)). The SDS detergent solubilizes the phospholipids and proteins of the cell membrane resulting in cell lysis and the release of the cells contents. The high concentration of sodium hydroxide denatures the genomic and plasmid DNA, as well as cellular proteins. The cellular DNA becomes linearized and the strands are separated, where as the plasmid DNA is circular and remains topologically constrained (the two strands, although denatured remain together).

Finally, a neutralization buffer of potassium acetate is added to neutralize the strong alkaline conditions. The addition of potassium acetate results in a high salt concentration that leads to the formation of a white precipitate that consists of SDS, lipids and proteins. In addition, the neutralization of the solution allows the renaturation of DNA. The large chromosomal DNA is captured in the precipitate, where as the small plasmid DNA remains in solution. The precipitate and chromosomal DNA is removed by centrifugation.

Following centrifugation, the soluble plasmid DNA can be purified from the solution by various techniques. The most common is to precipitate the DNA with alcohol (ethanol or isopropanol) or high salt (ammonium acetate, lithium chloride, sodium chloride or sodium acetate). Another method is to bind the DNA to a solid support, such as glass fibers or silica. At high salt concentration and neutral or low pH, DNA molecules have a high binding affinity for these supports, allowing for the easy capture and subsequent elution of the DNA.

PROCEDURE

1. Each student labels two 1.5ml Centrifuge tubes with his or her name.

2. Transfer 1.5ml overnight culture into labeled 1.5ml centrifuge tube.

3. Centrifuge at high speed for 3-5 minutes to pellet bacteria. Pour off the supernatant.

4. Add 200µl Cell Suspension Solution and mix the contents by flicking the tube or pipetting several times.
   This solution contains Tris (pH 7.5), and EDTA (ethylenediaminetetraacetic acid). The basic pH helps to denature the DNA and the metal ion chelator, EDTA, stabilizes the cell membrane by binding the divalent cations of Mg^{2+} and Ca^{2+}. RNase can also be added at this stage to degrade the RNA when the cells are lysed.

5. Incubate the vial on ice for 15 minutes.

6. Add 200µl Lysis Buffer and mix the contents by gently inverting the tube 4-5 times.
   This solution contains sodium hydroxide and SDS (sodium dodecyl sulfate). The sodium hydroxide denatures the plasmid and chromosomal DNA into single strands. SDS, an ionic (charged) detergent dissolves the phospholipids in the membrane causing lysis and release of the bacteria contents, including the DNA, into the solution.

7. Add 200µl Neutralization Buffer and mix the contents by inverting the tube 4-5 times.
   This is a potassium acetate solution. The potassium acetate causes the precipitation of a SDS-protein complex as a white precipitate, consisting of SDS, lipids and proteins. In addition, the potassium acetate neutralizes the solution

allowing the renaturation of the DNA. The large chromosomal DNA is captured in the precipitate, whereas the small plasmid DNA remains in solution.

8. The tube is then centrifuged for 10 minutes at high speed (>5,000xg).

9. Transfer the clear liquid, or supernatant, to a fresh labeled 1.5ml tube. This can be stored for up to a week at 4°C.

10. Alcohol Precipitation: To precipitate the plasmid DNA, add 480µl (0.8 volumes) Precipitation Solution (isopropanol) to the supernatants from step 9.
Ethanol can be used instead of isopropanol and should be used at 2.5 volumes.

11. Mix the tubes by inverting 5 times. Place the tubes at -20°C for 10 minutes.

12. To pellet the plasmid DNA centrifuge at full speed for 15 minutes.

13. After centrifugation, examine the tubes for a small white pellet of plasmid DNA. Pour off the supernatants.

14. Add 300µl DNA Wash (70% isopropanol) to the pellets to wash away any excess salt. Centrifuge the tubes at full speed for 5 minutes.

15. Carefully remove the supernatants with a pipette and leave the tubes open on your bench to allow all residual alcohol to evaporate.

16. Once all alcohol has evaporated, add 50µl TE buffer to the pellets. Wait 2 minutes then vortex to resuspend the DNA.
This is your purified plasmid DNA.

17. The DNA can be run on an agarose gel to visualize the DNA or can be subjected to restriction digestion analysis and then agarose electrophoresis to check the plasmids.