II. Preparing the Plasmid DNA

1. Add 0.2 mls of Quick lysis solution to each tube and resuspend the pellet in the solution by gently shaking the tubes. This solution contains lysozyme which degrades the bacterial cell wall and initiates bacterial cell lysis.

2. Incubate the tubes at room temperature for 5 minutes.

3. Add 0.4 mls of SDS-NaOH to each tube, shake gently until the contents are mixed thoroughly, and then incubate on ice for 10 minutes. This solution should become clear and viscous. The SDS-NaOH contains the detergent sodium dodecyl sulfate which dissolves the bacterial membranes and causes the final stages of cell lysis. The sodium hydroxide in the solution denatures the DNA.

4. Add 0.3 mls of ammonium acetate solution to each tube. Mix gently until the contents are thoroughly mixed and then incubate on ice for an additional 15 minutes. The ammonium acetate and SDS produce a tangled network of long strands of bacterial chromosomal DNA and cell debris, and this matrix can be separated from the smaller plasmid DNA by centrifugation.

5. Centrifuge the tubes for 10 minutes at 5,000 x g. At this point, plasmid DNA and some of the cellular *E. coli* RNA are in the supernatant, and bacterial chromosomal DNA and proteins are in the pellet.

6. Carefully pour the supernatant into a clean tube and add 0.6 mls of isopropanol and shake the tubes to mix the contents until a uniform suspension is obtained. Incubate the tubes at room temperature for 10 minutes. Isopropanol will precipitate the nucleic acids.

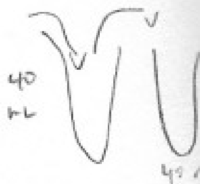
7. Centrifuge for 10 minutes at 5,000 – 8,000 x g.

8. Carefully pour off and discard the isopropanol making sure not to dislodge the DNA pellet in each tube. Add 0.6 mls of isopropanol to the pellet and centrifuge the tubes for 5 minutes at 5,000 – 8,000 x g. The DNA is visible as an opaque layer on the bottom of the tubes. Invert the tubes and allow the DNA pellet to dry for 15 minutes or longer. The tubes can be stored in the refrigerator until the next lab session ~~or you may proceed on~~.

Lab session 2

I. Preparing the DNA samples

1. Add 20 ul of water to each tube to dissolve the DNA. Combine both tubes into one to give a total volume of 40 ul.



2. Number two small minifuge tubes 1 and 2.

3. Place 20 ul of distilled water into tube #1 and 20 ul of the EcoRI buffer into tube #2.

4. Add 10 ul of your plasmid to each tube and tap the tubes with the tip of you index finger to mix the contents of the tubes.

5. Incubate the tubes for 50 minutes at 37°C.

6. At the end of the incubation period, add 10 ul of electrophoresis sample buffer to each tube. Incubate the tubes for 5 minutes at room temperature. This sample buffer contains Rnase which will destroy RNA in the samples.

II. Electrophoresis

1. Prepare a 1.2 % agarose gel.

2. ~~Add 70 ul of sample buffer to the pUC18 DNA tube.~~

3. Load 10-15 ul of the following samples into the sample wells of the agarose gels.

Sample well	Sample
1	DNA sample tube #1
2	DNA sample tube #2
3	Plasmid pUC18 DNA

4. Electrophorese at 100V until the tracking dye has migrated to within 2 mm of the positive end of the gel.

5. Remove the gels and stain with methylene blue. After 24 hours, the gels are to be destained with water.

Study questions

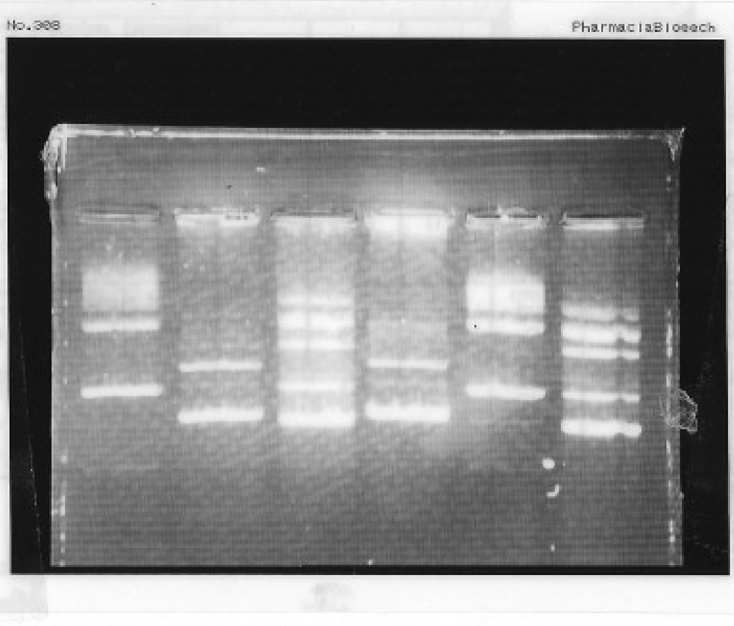
1. Describe the stained nucleic acid patterns you observed on your gels.

2. The SDS-ammonium acetate step and Rnase in the sample buffer serve to remove bacterial nucleic acids. Why do you think these steps were necessary? Discuss you answer in terms of the electrophoretic patterns you might have observed had these steps been omitted.

3. Explain the electrophoresis picture attached.

Wells

1	2	3	4	5	6
DNA #1	DNA #2	Plasmid pUC18 DNA	DNA #2	DNA #1	Plasmid pUC18 DNA



Wells 1 and 5 are the same.

Wells 2 and 4 are the same

Wells 3 and 6 are the same.