# Plasmid DNA Isolation

Prepare the necessary solutions on page 3 before beginning.

In a sterile flask containing 400ml of LB broth w/AMP grow an overnight culture of bacteria in a 37°C shaking water bath (or whatever environment your bacteria require.)

**Next Day:**

Pour the culture into a 450ml centrifuge bottle. Balance the bottles and centrifuge in the Sorvall Centrifuge for 20 minutes at a speed of 4000 rpm.

Pour off the supernatant and allow to drip for just a few seconds on some paper towels. At this step the pellet can be frozen and stored at -20°C. Resuspend the pellet in 10mls of Solution 1 (located at 4°C.)

Solution 1
50mM Glucose
10mM EDTA
25mM Tris pH 8.0

Add 20mg of Lysozyme solid. Mix very well making sure there are no solid pieces remaining. Allow to sit on ice for 15 minutes. During this time prepare Solution 2.

Solution 2
.2N NaOH (2mls of 10N NaOH/ 100ml solution)
1% SDS (10mls from 10% stock SDS/100ml solution)

After incubation add 20mls of Solution 2. It is very important to mix the samples very well. *Don't swirl. Use light flicking, like dropping solution of air. Add 10mls of Solution 3, previously made.

Solution 3
3M KOAc

After adding Solution 3 gently mix the solution. Don't shake, but swirl this time. When mixed well the yellow should disappear and white stringy pieces should appear. Allow to sit on ice for 15 minutes.

Spin the sample in the Sorvall Centrifuge for 10 minutes at 3500 rpm. When spun down transfer the supernatant to a 50ml centrifuge tube by pouring the solution through a funnel covered with gauze. Do not allow the white pieces to enter the flask. Fill the remaining amount of the tube with isopropyl alcohol (stored at -20°C.) Sit on ice for 30 minutes. Spin for 10 minutes at 3500 rpm.

Pour off the supernatant, leaving the pellet. Drain as much of the supernatant as possible, taking care not to lose the pellet.

Resuspend the pellet from one 400ml prep in 5mls of TE (10mM Tris/ 1mM EDTA.) If there is more than one pellet for a 400ml culture, resuspend one pellet and then use that liquid to resuspend the other. When the pellet is resuspended completely,

add 7.73g of Cesium Chloride to each sample. *Caution: Do not add Cesium Chloride if the pellet is not completely resuspended. The Cesium Chloride will take a while to dissolve. Pipet up and down constantly until there is no Cesium Chloride out of solution. If there is any Cesium Chloride out of solution it could ruin the Ultra Centrifuge rotor.

Transfer the sample into a Beckman Coulter 8.9ml Ultra Centrifuge tube. Add 350µl of Ethidium Bromide to each tube. *BE SURE TO WEAR GLOVES!! Ethidium Bromide is a carcinogen! Fill each tube to the neck with TE. Place the black stoppers in the top of the tube. Weigh each tube and match balanced pairs with .03g. Place the yellow caps on top of the tubes and place balanced pairs in the rotor. Centrifuge overnight at 55,000rpm at 23°C for at least 12 hours.

**Next Day:**

After the first run is complete remove the tubes carefully using the removal tongs. Be very careful or the bands will be disrupted. To remove the bands you will need the following:

Ring stand with claw clamp
26 gauge needle for each sample
1cc syringe for each sample

Place the tube in the clamps and insert a needle just below the band (Sometimes the band is very faint.) Remove the caps on the tube and pull out the band with the needle and syringe. You must be careful or the sample will come out around the needle hole. Place the sample in another 8.9ml ultra centrifuge tube. You can combine the samples with the same plasmid in the same tube at this point and fill the tube with a solution of CsCl/TE which has been previously prepared. Weigh each sample and match balanced pairs within .03g again and spin for 5-6 hours at 55,000 rpm.

When the run is complete follow the above instructions for removing the bands. Place the bands in a 50ml tube and add an equal volume of TE and mix well. Add a volume of Isoamyl alcohol equal to that of the band and the TE combined. Shake very well and then allow the phases to separate. Pipet off the top pink layer and repeat the extractions until the top layer is clear.

Prepare pieces of dialysis tubing by rinsing very well in distilled water. Place a clip on the bottom and carefully dispense the extracted band into the tubing. Clip the top and place the sample in a 1L beaker containing a solution of:

10mls of 1M Tris
2mls of .5M EDTA pH 8.0
1L dd$H_2O$

Dialyze for at least 6 hours at 4°C with a stir bar. Pour off old solution and repeat process again. After the second dialysis remove the liquid and pipet into a 50ml tube. Add 1/10 volume 5M NaCl and 2X volume of 200 proof (100%) Ethanol. Mix very well and centrifuge the samples in the Sorvall centrifuge for 15 minutes at 4000 rpm at 4°C. When spin is complete remove tubes and pour off the supernatant leaving the pellet behind. Rinse with 95% Ethanol and resuspend the pellet in 100µl of dd$H_2O$ (depending on size of the pellet.)

# Solutions to prepare before beginning

1. 10M NaOH
    a. 200g NaOH
    b. 450ml $H_2O$, bring up to 500ml
2. 1M HCl
    a. 91.4ml $H_2O$
    b. 8.6ml HCl
3. 1M TrisCl pH 8.0
    a. 60.5g TrisCl
    b. 400ml $H_2O$
    c. 29.2ml .1M HCl
    d. Bring volume to 500ml
4. 1M TrisCl pH 7.5
    a. 60.5g TrisCl
    b. 400ml $H_2O$
    c. 40.3ml .1M HCl
    d. Bring volume to 500ml
5. 500mM EDTA pH 8.0
    a. 93.05g EDTA
    b. 350ml $H_2O$
    c. 25ml 10M NaOH
    d. Bring volume to 500ml
6. 500mM EDTA pH 7.5
    a. 93.05g EDTA
    b. 350 ml $H_2O$
    c. 15ml 10M NaOH
    d. Bring volume to 500ml
7. 500mM Glucose
    a. 4.505g Glucose
    b. 50ml $H_2O$ total volume
8. Solution 1
    a. 10ml 500mM Glucose
    b. 2ml 500mM EDTA pH 8.0
    c. 2.5ml 1M Tris pH 8.0
    d. 85.5ml $H_2O$
    e. Autoclave and store at 4°C
9. 10% SDS
    a. 1:1 dilution of 20% SDS into $H_2O$
10. TE
    a. 5ml 1M Tris pH 7.5
    b. 1ml 500mM EDTA pH 7.5
    c. Bring volume to 500ml with $H_2O$
11. CsCl Solution
    a. 86.8g CsCl
    b. Bring volume to 100ml with TE

12. 10mg/ml Ethidium Bromide
    a. 0.3g EtBr
    b. 30ml $H_2O$
13. 5M NaCl
    a. 29.22g NaCl
    b. Bring volume to 100ml with $H_2O$
14. Solution 3
    a. 60ml 5M potassium acetate (49.07g potassium acetate in 100ml $H_2O$)
    b. 11.5ml glacial acetate
    c. 28.5ml $H_2O$