MINI PREP FOLLOWING MANIATIS PROTOCOL

Overnight Cultures

1. Add 5ml of LB broth to a 15ml conical tube.
2. Add 20ul ampicillin [stock 25mg/ml] [final 100ug/ml]
3. Pick a colony from the plate with loop and shake loop in LB amp broth.
4. Repeat for all plates that DNA will be obtained from.
5. Place all the tubes in the warm room and shake at 250rpm's overnight.

Maniatis Protocol (alkaline Method)

1. Pipet 1.5ml of each overnight culture into a 1.5ml microcentrifuge tube.
2. Spin the tubes down for 3 minutes at 13,200rpms at 4°C.
3. Remove the medium by steril aspiration, leave the pellet as dry as possible.
4. Resuspend the pellet in 100ul of Solution I and sit at room temperature for 5 minutes.
5. During the 5 minute incubation above, make solution II.
   1ml of 1N NaOH
   500ul of 10% SDS
   Q.S. to 5mls.
6. After the 5 minute incubation, add 200 ul of solution II and mix by inverting five or so times (DO NOT VORTEX) and then incubate on ice for 5 minutes.
7. Add 150ul of Solution III to each tube and vortex upside down gently for 10 seconds. Then incubate on ice for 5 minutes.
8. Centrifuge the tubes for 5 minutes at 4°C at 13,200rpms.
9. Transfer the supernatant to a new tube.
10. Do a 1:1 phenol-chloroform extraction, placing the supernatant in a new tube again.
11. Add 2 volumes of cold ethanol and vortex. Let stand at room temperature for 10 minutes and then centrifuge for 15 minutes at 4°C at a speed of 13,200rpms.
12. Aspirate all the supernatant off and get as dry as possible. Set in a hood until dry and then resuspend the pellet in 40ul of dH_2O.

    NB: A wash with 70% ethanol can be done before drying and resuspension in water. Some of the pellet will be lost though.

13. Vortex and let the pellet resuspend overnight if able. Spec the samples.