DNA Max-Prep

1. Inoculate 500ml TB+Amp with either 1ml stock bacterial culture or transformation. Allow culture to grow overnight at 37oC while shaking at 250 RPM.
2. Pellet bacterial cells with a 3000 RPM spin for 7 minutes at 4oC. Discard supernatant. Place pellets on ice. Add 25ml Solution I* and resuspend cells by placing tube in shaking incubator for about 5 minutes or until cells are resuspended. Place cells on ice.
3. Add 50ml Solution II and mix by shaking. Set cells on ice for 10 minutes.
4. Add 37.5ml Solution III and mix by shaking. Set cells on ice for 10 minutes.
5. Spin lysed cells at 7000 RPM for 15 minutes at 4oC.
6. Filter supernatant through a Kim-wipe and collect in a fresh centrifuge tube. Add 60ml (0.6 volume) Isopropanol and mix by inverting tube several times. Set at room temperature for 20 minutes.
7. Pellet DNA with a 7000 RPM spin for 20 minutes at room temperature. Discard supernatant. Dry out pellet slightly.
8. Add 3ml 1X TE to DNA pellet and resuspend in shaking incubator.
9. Once DNA is in solution, measure the volume of each DNA sample. Add 1.08/ml CsCl to each sample and dissolve by swirling tube gently.
10. Test the sample's refractive index with the refractometer. Adjust with either 1X TE or CsCl until the refractive index equals 1.38.
11. Transfer the DNA into 6ml ultracentrifuge tubes. Add 100ul Ethidium Bromide. Top off the tube with standard CsCl solution (1.08g/ml).
12. Crimp the tubes closed and spin for 4 hours at 65K at 22oC or overnight at 60K at 22oC.
13. Remove tubes from rotor. DNA should be banded in middle section of ultracentrifuge tube, red in color. Use 18 gauge needle and 5c syringe to extract the DNA band from the tube. Transfer the DNA to a 15ml tube.
14. Wash DNA 5 times with 2ml water saturated butanol, aspirating the top layer between washes.
15. Transfer the bottom aqueous layer containing the DNA to dialysis tubing and dialyses for 1 hour in 4L 1X TE. Dialyses 2 more hours in fresh 1X TE.
16. Transfer DNA solution to 30ml glass tubes. Measure the total volume of each sample and add 1/10 total volume 3M NaAcetate and 2 ½X total volume cold 100% ethanol. Parafilm the openings and place at –20oC overnight.
17. Pellet precipitated DNA with a 6000 RPM spin for 20 minutes at 4oC. Discard supernatant. Allow pellet to dry slightly to air. Resuspend DNA in an appropriate amount of 1X TE, depending on the size of the DNA pellet (500-1000ul).
18. Set DNA samples at 37oC of 30 minutes to ensure resuspension.
19. Take spectrophotometer readings and calculate concentration of DNA samples. Run an analytical gel to see how prep DNA looks.

*Volumes of the solutions added are based upon a 500ml overnight bacterial culture; Adjust volumes accordingly depending on the size of the overnight culture.