DNA Mini-Prep

1. Inoculate 2ml TB or LB + 100ug/ml Amp with a single transformed bacteria colony. Incubate overnight at 37°C while shaking at 250 RPM.
2. The next day, transfer 1.7ml of overnight culture to 1.7ml a microcentrifuge tube. Pellet cells with a 5000 RPM spin for 1 minute at room temperature. Aspirate off supernatant.
3. Add 100ul Solution I and resuspend cells by vortexing. Place cells on ice.
4. Add 200ul Solution II. Vortex thoroughly. Place cells on ice for 10 minutes.
5. Add 150ul Solution III. Vortex thoroughly. Place on ice 10 minutes.
6. Pellet cell debris with a 15,000 RPM spin for 12 minutes at room temperature. Transfer supernatant to a new microcentrifuge tube.
7. Add 400ul Phenol:Chloroform. Vortex thoroughly (0.5-1 minute). Spin at 15,000 RPM for 3 minutes at room temperature.
8. Transfer aqueous (top) phase to a new microcentrifuge tube. Add 400ul Chloroform:Iso Amyl Alcohol. Vortex thoroughly (0.5-1 minute). Spin at 15,000 RPM for 3 minutes at room temperature.
9. Transfer aqueous (top) phase to a new microcentrifuge tube. Add 1ml cold 100% EtOH. Mix by inverting the tube several times.
10. Snap freeze on dry ice or at –140°C for atleast 30 minutes.
11. Pellet precipitated DNA with a 15,000RPM spin for 15 minutes at 4°C. Aspirate off supernatant, being careful not to disturb the pellet.
12. Wash DNA pellet by adding 1ml 70% EtOH and spinning at 15,000 RPM for 5 minutes at room temperature. Make sure tube is in the same orientation as the previous spin.
13. Aspirate off supernatant. Dry pellet in speed vac (about 10 minutes). Resuspend pellet in 25ul 1X TE + 1ug/ml RNase. Incubate resuspended DNA at room temperature or 37°C for 30 minutes followed by 10minutes at 65°C.