DNA Mini-Prep V2

1) Inoculate 3ml TB or LB + 100µg/mL Amp (or similar selection) with a single transformed bacteria colony. Incubate overnight at 37°C while shaking at 250 rpm.
2) Transfer 1.7mL of the overnight culture to a 1.7mL microcentrifuge tube. Pellet cells with a 5,000 rpm spin for 1 minute at room temperature. Aspirate supernatant.
3) Add 100µL Solution I and resuspend cells by vortexing. Place cells on ice for 3-4 minutes.
4) Add 200µL Solution II. Vortex thoroughly. Place cells on ice for 10 minutes.
5) Add 150µL Solution III. Vortex thoroughly. Place cells on ice for 10 minutes (OK to leave longer).
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 25µL 1X TE + 1µg/mL RNase. Incubate at room temperature or 37°C for 30 minutes followed by 10 minutes in the 55°C water bath.
8) Add 400µL Phenol: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 and repeat Step 8.
10) Transfer aqueous (top) phase to a new microcentrifuge tube. Add 40µL 3M NaAc. Add 1mL cold 100% EtOH. Mix by inverting the tube several times.
11) Snap freeze on dry ice or in -80°C for at least 30 minutes.
12) Pellet precipitated DNA with a 15,000 rpm spin for 15 minutes at 4°C. Aspirate off supernatant, being careful not to disturb the pellet.
13) Wash DNA pellet by adding 1mL 70% cold 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.
14) Aspirate supernatant. Dry pellet in speed vac (until pellet is dry). Resuspend pellet in 25µL ddH2O. Store at 4°C.