Maxiprep Protocol:6/19/2017

1. Harvest bacterial culture after 12-16 hours of growth by centrifuging at 6,000 xg for 15min at 4°C and freeze at -20°C overnight.

2. Thaw frozen pellet on ice for approximately 10 min and completely re-suspend in 10ml P1 buffer. While thawing place P3 buffer on ice.

3. Add 10ml P2 buffer, mix by inverting sealed tube 4-6 times, incubate at room temperature for up to 5min.
   a. During incubation screw cap onto outlet nozzle of the QIAfilter cartridge.

4. Add 10ml pre-chilled P3 buffer, mix by inverting sealed tube 4-6 times, pour lysate into the barrel of QIAfilter cartridge and incubate at room temperature for up to 10min. Do not insert plunger!
   a. During incubation equilibrate the QIAGEN-tip by applying 10ml QBT buffer and allowing the column to empty by gravity flow.

5. Remove cap from QIAfilter cartridge, carefully insert plunger, and filter the cell lysate into the equilibrated QIAGEN-tip. Allow lysate to enter the resin by gravity flow.

6. Wash the QIAGEN-tip 2x with 30ml QC buffer, allowing column to empty completely through gravity flow.
   a. While waiting, put 10.5ml room-temperature isopropanol into a 50ml conical tube.

7. Elute DNA with 15ml QF buffer into 50ml conical tube containing isopropanol by gravity flow.
   a. Allow solution to sit for 1-2 hours

8. Centrifuge solution at 4°C for 30min at 3,500 xg.
   a. If no pellet is visible, spin for another 30 min at 3500 xg.

9. Decant supernatant, re-suspend pellet in 1ml 70% ethanol in eppendorf tube and spin at 15,000 xg for 10min.

10. Air dry the pellet for 5-10min and re-dissolve in 200ul ddH_2O.
    a. Can be helpful to use a slightly alkaline buffer (TE,etc.) or heat elution buffer ~50C