

Plasmid Preps

- 1) Spin your cultures of cells in the clinical centrifuge at 5000g for 5 min. Pour the supernatant in the sink and 'dab' the tube upside down on paper towels to remove any excess supernatant.
- 2) Add 250 μ l of Buffer P1 to the cells and pipet up and down to mix.
- 3) Transfer the resuspended cells into a **labeled** microfuge tube.
- 4) Add 250 μ l of Buffer P2 and gently invert tubes 4-6 times. Solution should become viscous and slightly clear.
- 5) Add 350 μ l of Buffer N3 and invert the tubes immediately but gently 4-6 times. The solution should become cloudy.
- 6) Centrifuge in a microfuge at maximum speed for 10 min. During centrifugation, place a Qiaprep spin column in a 2 ml collection tube and **label the columns** (not the tubes).
- 7) Apply the supernatants from step 6 to the labeled Qiaprep column by decanting (pouring).
- 8) Centrifuge 30-60 seconds at maximum speed and discard the flow-through.
- 9) Add 500 μ l of Buffer PB to the column and centrifuge 30 seconds. Discard the flow-through.
- 10) Add 750 μ l of Buffer PE and centrifuge 30-60 seconds. Discard flow-through.
- 11) Put the column back in the same tube and spin again for 1 minute to remove residual buffer.
- 12) Place Qiaprep column in a clean 1.5 ml **labeled** microfuge tube. Add 50 μ l of sterile water to the center of each column, let stand for 1 minute, and centrifuge for 1 minute.
- 13) Give the flow through to the TAs for storage until Thursday.