Qiagen Maxiprep Protocol

Night before: for each sample, make 200 ml LB and autoclave in a 1L flask. Let cool, add 200Λ 50 mg/ml ampicillin, add picked colony, and shake o/n @ 37°C.

1. Pour broth into plastic centrifuge bottle. Spin for 15' at 5000 rpm.

2. Pour off supernatant; resuspend bacterial pellet in 10 ml **Buffer P1** (this should already be cold, as its stored in the refrigerator with RNAse). Transfer resuspended pellet solution to a plastic centrifuge tube.

3. Add 10 ml **Buffer P2**, mix gently, and incubate at room temperature for 5 minutes.

4. Add 10 ml of cold **Buffer P3**, mix immediately but gently by inversion, and incubate on ice for 20 minutes.

5. Spin for 30' at 12,000 rpm @ 4 °C. During the last 10' of the spin, equilibrate **Qiagen-tip 500** column by adding 10 ml **Buffer QBT**. Allow column to drain.

6. Decant supernatent 2X in Falcon 50ml tubes. Remove remaining chunks with pipet.

7. Add decanted supernatent to column. Allow to flow through.

8. Wash column 2X with 30 ml **Buffer QC**.

9. Elute DNA by adding 2 x 5 ml **Buffer QF**. Catch eluate in fresh plastic centrifuge tube.

10. Precipitate DNA with 7 ml room-temperature isopropanol. Spin for 30' at 12,000 rpm @ 4°C.

11. Pour off supernatent; add 5 ml 70% ethanol; spin 5' at 12, 000 rpm @ 4 °C.

12. Pour off supernatent; respin for 5' at 12, 000 rpm @ 4°C and remove remaining drops with vacuum.

13. Air-dry for 5'; resuspend in 500 µl dH_20 or TE, transfer to an eppendorf. Respin for 5' at 5,000 rpm if necessary to get remaining DNA solution.

14. Restriction digest; test on gel.