MAXIPREP OF PLASMIDS FROM *E. COLI* USING WIZARD MAXIPREP KIT

Day 1

1. Inoculate 200 ml of medium containing appropriate antibiotic with *E. coli* strain carrying the plasmid of interest; incubate overnight at 37°C
2. Make up stocks of TE (10 mM Tris·HCL, pH 8; 1 mM EDTA, pH 8); 80% ethanol; Be sure to have some isopropanol and cheesecloth available; also make certain that the Wash Buffer* from the maxiprep kit has had ethanol added

Day 2

3. Centrifuge culture for 5 min. at 6 000 rpm in GSA rotor; discard supernatant
4. Resuspend pellet completely in 15 ml Resuspension Buffer*
5. Add 15 ml Lysis Buffer* while swirling the suspension
6. Once the cells have lysed (typically 1-20 minutes, the suspension will become more translucent and "goopy") add 15 ml Neutralization Buffer* while swirling
7. Immediately centrifuge for 10 min at 6 500 rpm in GSA rotor; while this is spinning, prepare some more centrifuge bottles (one per sample) by pipetting into each of them ~27 ml isopropanol, and placing a square of cheesecloth (or other filtering material) over the opening of the centrifuge bottle
8. Pour supernatant through the cheesecloth into the centrifuge bottles containing the isopropanol (first you may want to "dent in" the cheesecloth to form a pocket into which you will pour the supernatant)
9. Mix well and centrifuge 15 minutes at 8 000 rpm in GSA rotor
10. Pour off supernatant and blot excess liquid away onto paper towel; add 2 ml TE to centrifuge bottle to rehydrate/resuspend the pellet (pellet may not be visible, but don't let this deter you)
11. Add 10 ml well-mixed Maxiprep Resin* to the sample and mix well by swirling (caution, this contains guanidine, which is pretty caustic)
12. Affix Maxiprep Column* to the vacuum manifold (one per sample) and pour the resin/DNA onto the column; apply the vacuum
13. As the sample is drawn through the column, pipette 13 ml of Wash Buffer (with ethanol) into the centrifuge bottle, swirl to rinse out whatever resin/DNA remains and then pour this onto the Maxiprep Column after the first material has passed through

14. Using another 12 ml of Wash Buffer* again rinse out the centrifuge bottle and pour this onto the Maxiprep Column as well
15. Pipette 5 ml 80% ethanol onto the column as a final wash; let the column air dry with the vacuum still pulling for 2-5 minutes
16. Remove the Maxiprep Column from the manifold, place it into the 50 ml blue-capped tube and centrifuge for 5 min at ~4 500 rpm in the clinical (swinging bucket) centrifuge; while this is spinning, preheat some TE to 55°C-65°C
17. Remove column from centrifuge tube; pipette out any liquid that might be at the bottom; replace the column into the tube and pipette into the column 1.5-2 ml of prewarmed TE
18. After about 1 minute, centrifuge this again for 5 minutes in the clinical centrifuge (4 500 rpm)
19. DNA solution will have collected in the bottom of the centrifuge tube; transfer this to a smaller eppendorf tube or screw-capped tube and store at 4°C

*Supplied with Wizard Maxiprep Kit