PLASMID PREP (USING QIAGEN MAXI KIT)

Purpose: The purpose of this experiment is to isolate plasmid DNA from E-coli.

Preparation of Culture:

1. Broth is prepared using two 1000ml flasks filled with 300ml of dH20 each. Six LB tablets are placed in each flask (1 LB tablet per 50 ml of water).
2. Swirl mixture & autoclaved.
3. Flask is cooled to the touch (or in water bath if limited time)
4. Ampicillin frozen stock (freezer 5S-19) is 25 mg/ml; want 100 ug/ ml final conc. Amount of Ampicillin to add was determined using C1V1=C2V2. Ex: add 1.2 ml ampicillin to cooled 300 ml LB broth just before use.
5. Broth is inoculated with bacterial glycerol from -80°C freezer. 200-300 ul of bacteria is added to each flask.
6. Flasks were placed in the Microbiology's 37° room overnight to stir @ ~300rpm.

Procedure:

1. Transfer maximum of 300ml of overnight culture to each 500ml centrifuge bottle(s).
2. Bottles were balanced & spun at 6000 rpm at 4 degrees Celsius for 30 min .
3. After centrifugation, cell-free supernatant was carefully poured back into the original culture flasks and re-autoclaved to kill the remnants of bacteria present. The re-autoclaved culture supernatant can then be safely poured down the sink.
4. Meanwhile, resuspend each pellet in 10 ml of chilled Qiagen Buffer P1 (should already contain LyseBlue and RNase)
5. Mixture was triturated well to remove pellets from side of bottle(s) and to ensure no clumps remain. Transfer each resuspended bacterial pellet(s) to a separate 50 ml round bottom tube (NOT disposable)
6. Add 10 ml of Qiagen Buffer P2 (room temp) to each tube, cap quickly and invert many times until the entire sample is blue. Incubate the mixture at room temp. for 5 min.
7. Add 10 ml of chilled Qiagen Buffer P3 (deli 5S-19) to each tube, cap quickly and inverted vigorously until the entire sample is white. Incubate the tubes on ice for 20 min.
8. Tubes were balanced using dH2O as needed
9. Spin at 13000rpm for 30 min. at 4 degrees Celsius.
10. Supernatant is transferred to new clean 50ml tubes & spun again at 13000rpm for 20 min. at 4 degrees Celsius.
11. Qiagen Maxi filtration columns (Qiagen tip-500 columns from Maxi kit) are setup over a 250 ml Erlenmeyer flask ("waste collection flask")

12. 10 ml of Qiagen Buffer QBT is added to each column and allowed to drip through by gravity.
13. The Supernatant (contains the plasmid) is then poured onto the column (300 ml max vol/Maxi column) and allowed to drip through into same waste collection flask.
14. After all the supernatant has dripped through, wash the column with 2 x 30ml of Qiagen Buffer QC and allowed to drip through into waste flask. May now discard the contents of the flask.
15. Set column over 50ml centrifuge bottle
16. 15ml of Qiagen Buffer QF is pipetted onto the column and the eluate is collected in the 50ml conical tube.
17. Add 10.5ml of Isopropanol (2-propanol) at RT, inverted several times to mix.
18. Tubes are spin at 12000rpm for 30 min. at 4 degrees Celsius.
19. Carefully decant Isopropanol
20. Pellet is washed with 5ml 70% ethanol
21. Spin at 12000rpm for 15 min. at 4 degrees Celsius.
22. Supernatant is aspirated off carefully.
23. Pellets are allowed to air dry under hood for ~30 min.
24. Resuspend pellet in 500ul of DNAse/RNAse free water.
25. Nano Drop Samples for concentration
26. Store the DNA @ -80°C