PROTOCAL FOR ISOLATING PLASMID DNA FOR AUTOMATED SEQUENCING

1. Pellet 1.5 ml aliquots of culture for 1min. (a total culture volume of 4.5 ml can be spun down per tube without changing volume in the procedure. This allows you to achieve a threefold increase in yield
2. Resuspend the bacterial pellet in 200ul of GET buffer thoroughly. (250ul).
3. Add 300ul of freshly prepared 0.2 N NaOH + 1% SDS. Mix the content of the tube by inversion .Incubate on ice for 5 mins.(500ul)
4. Neutralize the solution by adding 300ul of 3.0 M potassium acetate. pH 4.8. Mix by inversion .Incubate on ice for 5 mins.
5. Remove the debris by spinning in a microfuge at maximum speed for 10 mins at room temp. Transfer the supernatant in a clean tube.
6. Add RNAse A to a final conc. of 20ug/ml and incubate at 45’c for 1 hr.
7. Extract twice with chloroform
8. Add 400 ul of chloroform
9. Mix layers by inversion for 30 sec.
10. Centrifuge for 1 min to separate phase.
11. Transfer the upper layer to a clean tube.
12. **Add an equal volume of 100% isopropanol. Mix by inversion**.
13. Spin it in a microfuge at a maximum speed at room temperature for 10 mins .Remove
14. Wash the pellet with 500ul of 70% ethanol. Dry under vacuum.
15. Dissolve the pellet in 32 ul of deionised water; add 8.0 ul of 4M NaCl and then 40 ul 13% PEG 8000.
16. Mix thoroughly and leave the sample on ice for 20 mins.
17. Pellet the DNA by spinning in a microfuge for 15 mins at 2’C - 6’C.
18. Carefully remove the sup. Rinse the pellet with 500ul of 70 % ethanol (twice).
19. Resuspend the dried pellet in 20ul of deionised water. Store at -20’C.

**PLASMID DNA ISOLATION BY KIT OR PCR DNA**

1. Make volume up to 100ul with deionised water.
2. Add 250ul ethanol and 10ul of 3M Na-Acetate (pH5.2)
3. Spin at 12K for 15mins at room temp.
4. Wash the pellet twice with 70% ethanol. Dissolve in 20ul of deionised water.

(***The kit-purified DNA should be precipitated with room temperature Ethanol after incubating at RT for 10 min (not in ice))***.

**GET Buffer**

50mM Glucose

10mM EDTA

25mM Tris pH 8.0

Stock SDS -10%

Stock NaoH – 2N

1% SDS – 1/10 x 8000 = 800ul (10%)

0.2 N NaoH – 0.2/2x8000 = 800ul (2N NaoH)

Sterile MQ water = 6.400 ml

Total = 8 ml

**Required amount of template for sequencing:**

|  |  |
| --- | --- |
| **Template** | **Template Quantity** |
| PCR product : |  |
|  100-200 bp | 1-3 ng |
|  200-500 bp | 3-10 ng  |
|  500- 1000 bp | 5-20 ng  |
|  1000-2000 bp | 10-40 ng |
|  >2000 bp | 40-100 ng |
| Single-stranded | 50-100 ng |
| Double-stranded | 200-500 ng |
| Cosmid, BAC | 0.5-1.0 ug |
| Bacterial genomic DNA | 2-3ug |