Alkaline Lysis of Bacteria and Plasmid Prep

Mini prep (2~5 ml culture)

-Note: Follow the manufacture's instruction in using mini prep kit.

1. Inoculate a colony into 2 ml LB contains the appropriate antibiotics (30 μg/ml ampicillin, 15 μg/ml tetracycline, 30 μg/ml chloramphenicyl and 50 μg/ml kanamycin) and grow O/N at 37˚C under vigorous agitation.
2. Transfer 1.5 ml culture medium into 1.5 ml microcentrifugation tube.
3. Centrifuge at 5,000 rpm for 1 min at r.t.
4. Resuspend the pellet in 200 μl solution I.
5. Add 200 μl solution II, invert to mix and incubate on ice for 5 min.
6. Add 200 μl solution III and leave on ice for 10 min.
7. Centrifuge at 15,000 rpm for 10 min at r.t.
8. Extract plasmid DNA with equal volume of phenol/chloroform (1:1) and chloroform.
9. Add equal volume of isopropanol and 2 μl glycogen.
10. Centrifuge at 15,000 rpm for 10 min at 4C.
11. Remove supernatant, resuspend the pellet in 50 μl TE and 1 μl RNase and incubate fr 1 h at 37˚C.
12. Add 95 μl TE, 5 μl 20% SDS and 1 μl proteinase K and incubate O/N at 37˚C.
13. Extract plasmid DNA with equal volume of phenol/chloroform (1:1) and chloroform.
14. Precipitate plasmid DNA with ethanol.
15. Resuspend pellet in the appropriate buffer or H2O.

Cesium chloride gradient mega-prep DNA plasmid purification

1. Inoculate bacteria transformed plasmid into 450 ml LB contains the appropriate antibiotics (30 μg/ml ampicillin, 15 μg/ml tetracycline, 30 μg/ml chloramphenicol and 50 μg/ml kanamycin) and grow O/N at 37˚C under vigorous agitation.
2. Transfer culture into 450 ml tubes (Sorvall GS3 rotor) or 250 ml (Sorvall GSA rotor). Screw o-ring fitted caps on tightly and centrifuge at 5,000 rpm for 10 min at 4˚C.
3. Pour off supernatant into dilute chlorine solution.
   -Note: Pellet can be frozen at -20˚C for a stopping point.
4. Resuspend pellet in 18 ml of solution I and add 2 ml fresh lysozyme solution and incubate for 5 min at r.t.
5. Add 40 ml of solution II and mix thoroughly by gentle inversions. Incubate for 5~10 min at r.t.
6. Add 30 ml of cold solution III and shake thoroughly. Incubate on ice for 10 min.
   -Note: A flocculent white precipitate should form.
7. Centrifuge lysate at 4,000 rpm for 15 min at 4˚C. Allow rotor to stop without breaking.
8. Filter supernatant through 4 layers of cheesecloth into a 250 ml (Sorvall GSA rotor) plastic

bottle.

9. Add 0.6 volume isopropanol (54 ml) and mix thoroughly and incubate on ice for 10 min.
10. Centrifuge at 5,000 rpm for 15 min at r.t., carefully decant off all supernatant.
11. Rinse pellet with 70% EtOH and allow to dry.
12. Resuspend pellet in 4 ml TE buffer. Add 4 g $CsCl_2$ and dissolve.
13. Using a Pasteur pipette as a funnel, transfer to a 16 mm x 76 mm Backman quick-seal centrifuge tube. Fill tube to the line at the bottom of the neck with a1 g $CsCl_2$/ml TE solution. Add 50µl10 mg/ml ethidium bromide solution to the base of the sealing tube ensuring elimination of any trapped air bubbles. Keep sealing dry.
14. Weigh tubes. Ensure rotor-opposing tubes are with 0.02 g of each other.
15. Seal tube with Beckman quick-seal device. Squeeze tubes to ensure there is no leakage.

Buffers

Lysozyme solution -10 mg/ml lysozyme and 10 mM Tris pH 8.0

Lysozyme	1 g
1M Tris pH 8.0	1 ml
dH_2O	adjust to final volume of 100 ml

Solution I -50 mM Glucose, 25 mM Tris pH 8.0, and 10 mM EDTA

20% glucose	5 ml
1M Tris pH 8.0	2.5 ml
0.5 M EDTA pH 8.0	2 ml
dH_2O	adjust to final volume of 100 ml

Solution II -0.2 N NaOH, and 1% SDS

10 N NaOH	2 ml
20% SDS	5 ml
dH_2O	adjust to final volume of 100 ml

Solution III -5 M Pottasium acetate

5 M Pottasium Acetate	300 ml
Glacial Acetic Acid	57.5 ml
dH_2O	adjust to final volume of 500 ml