

LARGE SCALE PLASMID PREP

Yield: About 1 mg per liter of cells. Greatly varies with type of plasmid vector

Solutions:

Triton Lysis Buffer

(12 ml/liter cells):
50 mM Tris pH8
62.5 mM EDTA
0.4% Triton x-100

Wash Buffer

(50-75 ml/liter cells):
20 mM Tris pH 8
1 mM EDTA

Lysozyme Solution

(1.5ml/liter cells):
note: make fresh
Lysozyme to 10 mg/ml
in 250 mM EDTA

Sucrose buffer

(7.5 ml/liter cells)
25% Sucrose
50 mM Tris pH 8.0

EDTA

(1.5 ml/liter cells)
500 mM EDTA

TE (3 liters for 4 liters cells)

10 mM Tris 8
1 mM EDTA

6xProteinase K buffer

(5 ml/liter cells):
1.2 M LiCl
60 mM EDTA
60 mM Tris pH 8.0
1.2% SDS

Procedure:

(Note: put Ti60 in cold room, sign up for ultracentrifuge spins).

Day 1.

Starting with *E. coli* from a single colony grown on selective media, inoculate 5 to 10 ml LB containing antibiotic. Shake at 37°C overnight.

Day 2

1. Inoculate 10 ml Media with 1ml of overnight culture (1:10 dilution)
2. Shake 37°C about 3 hrs. (optional).
3. Inoculate 1 liter LB + antibiotic at 50*µg/ml with 5 to 10 ml of culture.
4. Shake at 37°C until O.D. 550 nm is about 1.0.
5. Add Chloramphenicol to 200 µg/ml (0.2g/liter).
6. Shake overnight 37°C.

Day 3

1. Collect cells in 500 ml centrifuge bottles in GS3 rotor at 4000 RPM, 10 min.
2. Pour off supernatants, autoclave or bleach and discard.

3. Pool and suspend cells in 50-75 ml cold wash buffer.
4. Collect 4K, 10 min in GS3 rotor.
5. Resuspend cells in 7.5 ml cold Sucrose buffer in polycarbonate centrifuge tube.
6. Add 1.5 ml lysozyme solution. Ice 5 min.
7. Add 1.5 ml 0.5 M EDTA, mix gently. Ice 5 min.
8. Add 12 ml Triton Lysis buffer, mix gently. Ice 10 min to 1 hour.
9. Spin 40K 1 hr in Ti 60 AT 4°C, or place in opaque plastic tubes and spin in SA-600, 10K for 90 min. at 4°C.
10. Remove supernatant to 2 30ml Corex tubes or one 50ml Corning tube.
11. Add 1/5 vol. 6x Proteinase K buffer and proteinase K to 100 *g/ml (about 1 tube of 250 µl 10 mg/ml).
12. Incubate 40 to 50°C for 2 hours.
13. Phenol/CHCl₃ extract once.
14. Remove aqueous phase to clean Corex tubes, add 2 volumes of Abs EtOH. Place in -80°C for 15 min
15. Spin in cold SA600 at 8K for 15 min. Allow pellets to air dry.
16. Resuspend in TE to final volume of 7ml.
17. Add 8g CsCl and 400 *l 10mg/ml EtBr. Place into quick seal tube and balance using paraffin oil.
18. Spin at 40K for 36 to 48 hours at 20°C.

Day 4

1. Remove DNA band w/5ml syringe and 18 gauge needle. (If bands are very faint, use only long wave UV to illuminate).
2. Extract 4 times with 1 to 2 vol. of isoamyl alcohol (Top phase is organic.)
3. Option 1: Place aqueous phase into dialysis tubing and dialyze against 3 changes of 1 liter TE with spinning. First for several hours at room temp. Then overnight in cold room. Finally change and continue to dialyze the duration of the next day in cold room.
4. Option 2: Dilute aqueous phase with 2 volumes H₂O, then EtOH precipitate with 6 volumes -20°C 100% ethanol and place at -20°C, overnight. Spin down EtOH precipitate 1 hr at 7,500 rpm in Corex tubes at 4°C. Resuspend in TE.
5. Place into well labeled tube. Make 1:100 dilution and check in spectrophotometer. (A₂₆₀ of 1 = 50 µg/ml DNA.)