OBJECTIVE: Genomic DNA isolation from *E.coli* DH5α cells.

Chemicals required: Tris-Cl, EDTA, sucrose, lysozyme, RNase, proteinase K, sodium dodecyl sulfate (SDS), tris saturated phenol, chloroform, sodium acetate, absolute ethanol, luria broth, NaOH, HCl.

Plastic ware/ glassware: measuring cylinder, autoclaved micro centrifuge tubes, 1.0 ml tips, 0.02ml-0.2 ml tips, enzyme tips, 100ml/250 ml flasks, reagent bottle, Test tubes. OakRidge tubes. Uvettes.

Equipment required: refrigerated centrifuge, vortex, UV spectrophotometer, water bath.

Recipe for solutions:

Luria broth: 1.0g in 50 ml (autoclaved)

Solution I: 10 mM Tris-Cl, 1 mM EDTA, 0.35 M sucrose. Adjust the pH to 8.0.

Lysozyme : 50 mg dissolved in 1.0 ml Milli Q.

RNase: 10 mg/ml stock

SDS: 2% w/v

Sodium acetate: 0.3 M. Adjust pH to 5.5.

TE buffer: 100 mM Tris-Cl, 10 mM EDTA. Adjust pH to 8.0

Procedure

Inoculate single colony of *E. coli* DH5α in 50 ml LB and grow it under appropriate conditions 37^0C, 180 rpm.

Harvest the cells (OD_{600}~ 1.2) at 12000 rpm for 1 min followed by washing with Milli Q water.

Resuspend the pellet in 12 ml of solution I and add 500 µl lysozyme (50 mg/ml stock) and 120 µl RNase (10mg/ml stock) and incubated at 37^0C for 1 hour.

Add 225µl of proteinase K (10mg/ml stock) to the suspension and incubate it at 55^0C for 6 hour* (usually for gram positive bacteria) with gentle inversion of tube in between.

(*in case of gram negative bacteria incubation is maximum extended upto 2-3 hour. The incubation time can be optimized by gradual clearing of suspension during incubation period.)

Add 12 ml of SDS solution (2% stock solution) and incubate it for 2 hour at 55^0C.

Extract the DNA by adding equal volume of phenol* : chloroform (1:1). (*phenol= tris saturated phenol, shake well before use.)

Centrifuge the suspension at 4000 rpm for 20 min at 10-12^0C.

Collect only upper aqueous layer with gentle pipetting.

To precipitate genomic DNA add 1/10 volume of 0.3M sodium acetate (pH 5.5), followed by addition of 2 volume of absolute ethanol.

Collect the DNA strands using sterile pipette tip in fresh microcentrifuge tube.

Wash the pellet with 70% ethanol and pellet is left for air drying.

Dissolve the dried pellet in ~5 ml of TE buffer.