

OBJECTIVE: Transformation of plasmid DNA in *E.coli* DH5 α cells.

Chemicals/ media required: Luria agar (HiMedia), Luria broth (HiMedia), appropriate antibiotic(HiMedia) ampicillin, ddH₂O/ milliQ.

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, 0.2 μ syringe filter, glass rod/spreader.

Equipment required: Heating bath

Recipe for media/antibiotic.

Luria Agar: 3.5g in 100 ml (autoclaved)

Luria broth: 2.0g in 100 ml (autoclaved)

Antibiotic: Ampicillin (100mg/ml). working concentration 100 μ g/ml.

PROTOCOL

- 1) Plasmid DNA (~1 μ g) is added to 100 μ l of chemically competent cells.
- 2) Mixture is tapped a little and incubated on ice for 30 min.
- 3) Thereafter, heat shock is given at 42°C for 90 second followed by incubation on ice for 5 mins.
- 4) 1 ml LB media is added to the cells and incubated at 37°C for 60- 90 min at 180 rpm in incubator shaker.
- 5) 100 μ l-200 μ l(depending on transformation efficiency of competent cells) of the culture is then spread on agar plate containing appropriate antibiotic.
- 6) Plate is incubated at 37°C overnight. Transformed colonies appear after 12-16 h of incubation.