

Isolate plasmid

Subcultured single colony into 10 ml of LB ampicillin

Grew overnight shaking 37C

Place 2 ml in microfuge tube

Spin 5 min at 5000 g

Decant

Add 200 ul **Cell resuspension solution**

Vortex

Add 200 ul **Cell lysis solution**

Add 200 ul **Neutralization solution**

Mix by inversion

Spin 3 min at 12,000 g

Place supernatant into new 1.5 ml tube

Add 1 ml **Resin suspension**

Mix by inversion

Attach column to 3 ml syringe

Pipette DNA/resin into syringe

Push gently through column

Remove column

Remove plunger

Reattach column and syringe

Add 2 ml **Column wash solution**

Push gently through column

Detach column

Remove plunger

Spin for 20 sec in 1.5 ml tube to dry resin

Place column in fresh 1.5 ml tube

Add 50 ul of **H2O** to column

Let sit for 1 min

Spin 20 sec at 12,000 to elute plasmid

Cell Resuspension Solution

50 mM Tris-HCl, pH7.5

10 mM EDTA

100 ug/ml RNAase A

TE Buffer 100 ml

10 mM Tris-HCl, pH 7.5 = 0.1211 g

1 mM EDTA 0.03362 g

Cell Lysis Solution 100 ml

0.2 M NaOH = 0.8 g

1% SDS = 1 g

Column Wash Solution 100 ml

200 mM NaCl = 1.17 g

20 mM Tris-HCl, pH 7.5 = 0.24 g
5 mM EDTA = 0.168 g
Dilute 1:1 with 95% ETOH

Neutralization Solution 100 ml

2.55 M KOAc, pH 4.8 = 25.03 g

Miniprep Resin 100 ml

10 mg/ml Diatomaceous Earth (Sigma D-5384) = 1.5 g
in 7.5 M Guanidine HCl