

# Plasmid Miniprep

2003-01-02 (from Chris Udell)

## ***Protocol***

1. Grow 3 mL overnight culture of E. coli containing your plasmid (in LB + selection (Amp, Kan, etc.).
2. Transfer 1.5 mL overnight culture to microfuge tube. Spin 1 min at max speed to pellet cells.
3. Discard super; resuspend pellet in **100  $\mu$ L TEG**. *Make sure pellet is completely resuspended by pipetting up and down several times..*
4. Add **200  $\mu$ L NaOH/SDS** lysis buffer. Mix gently but thoroughly by inverting tube 5-6 times. *SDS denatures proteins and NaOH denatures DNA.*
5. Before 1 min lysis time, add **150  $\mu$ L 3M KOAc** neutralization buffer and mix immediately but gently. *This neutralizes the mixture, allowing plasmid DNA to reanneal.*
6. Put on ice 5 min.
7. Spin 5 min to pellet cell debris and chromosomal DNA, as well as K-SDS precipitate.
8. Transfer super to new microfuge tube.
9. Extract with 400  $\mu$ L phenol:chloroform:IAA; keep **top (aqueous) phase (about 400  $\mu$ L)**.
10. Add **1/10 volume (40  $\mu$ L) of 3 M NaOAc** and **2 volumes (800  $\mu$ L) of chilled EtOH**. Mix, transfer to -20 °C and allow plasmid DNA to precipitate for at least 10 min. *Plasmid is safe at this step and can be left to precipitate overnight if necessary.*
11. Spin 5 min to pellet plasmid DNA. Wash pellet with 70% EtOH and spin again.
12. Discard super, dry pellet in 37 °C incubator for 10-15 min, OR SpeedVac.
13. Redissolve plasmid in 30-40  $\mu$ L ddH<sub>2</sub>O or TE buffer.

**Reagents:**

**TEG**

		[Final]
1.25 mL	1 M Tris (pH 8.0)	25 mM
1 mL	0.5 M EDTA	10 mM
1.13 mL	40% glucose (2.22 M)	50 mM
to 50 mL	<i>ddH<sub>2</sub>O</i>	

**NaOH/SDS lysis buffer**

		[Final]
1 mL	10 N NaOH	0.2 N
5 mL	10% SDS	1%
to 50 mL	<i>ddH<sub>2</sub>O</i>	

**3M KOAc neutralization buffer**

		[Final]
30 mL	5 M KOAc (14.7 g in 30 mL)	
5.75 mL	Glacial acetic acid	
to 50 mL	<i>ddH<sub>2</sub>O</i>	

**Other reagents:**

95% EtOH

70% EtOH

Phenol:Chloroform:IAA (25:24:1)

3M NaOAc (20.4 g in 50 mL, pH to 5.2 with glacial acetic acid)