PLASMID MINI PREP

Inoculate 5 ml YT + Amp with single bacterial colony. Incubate at 37 C over night with shaking.

Label 3 eppendorf tubes for each prep.

Pour 1.5 ml culture into eppendorf tube. Centrifuge for 30 seconds, aspirate and discard medium, refill tube and repeat. Store remaining culture at 4 C.

Resuspend cell pellet by vortexing in 200 μl TE + glucose at room temperature.

Add 200 μl freshly made 0.2 N NaOH/1% SDS. Mix by inverting the tube 5 times, then place on ice and open tube tops. Incubate 5 minutes (no longer).

Add 200 μl cold 3 M/5 M potassium acetate (pH approx. 4.8). Mix by inverting the tube 5 times, then incubate on ice for 5-10 minutes.

Spin in microfuge for 5 minutes.

Transfer to new tube containing 600 μl phenol: chloroform (2:1). Mix by vortexing, spin 2 minutes in microfuge.

Remove (top) aqueous phase to new tube.

Add 420 μl isopropanol, mix by vortexing, spin 5 minutes in microfuge.

Pour off supernatant and stand inverted tube on kimwipe to drain.

Add 800 μl 80% ethanol (-20 C), vortex and spin 5 minutes in microfuge. Pour off supernatant, drain tube, then dry pellet in speed vac.

Resuspend pellet in 50 μl TE (add boiled RNase A, 0.5 μl 10 mg/ml stock).

Store at -20 C. Use 2-5 μl for gel.

Solutions:

TE + glucose, 100 mlˇ

25 mM Tris-Cl (pH 8.0)	2.5 ml 1 M
10 mM EDTA	4.0 ml 0.25 M
50 mM glucose	0.9 g

0.2 N NaOH/1% SDS, 10 ml (make fresh)

2 ml 1.0 M NaOH
7 ml water
1 ml 10% SDS

3 M/5 M potassium acetate, 100 ml (store at 4 C)

60 ml 5 M potassium acetate
11.5 ml glacial acetic acid
28.5 ml water

Phenol:chloroform (2:1), store at 4 C

Add chloroform to Tris-saturated phenol (e.g. 15 ml chloroform to 30 ml phenol)
Vortex and spin 10 mins in clinical centrifuge. Store at 4 C. BE CAREFUL