**Plasmid Miniprep Protocols**

**Improved Alkaline Lysis Miniprep**

(adapted from Ahn et al., Biotechniques 29:466-468, 2000)

1) Grow overnight cultures in LB + appropriate antibiotic(s).

2) Transfer 1.5-3 mL of culture into an eppendorf tube and centrifuge at 5,000*g* for 1 min. Pipette off supernatant. (More than 1.5 mL can be pelleted in a single eppendorf tube by pelleting 1.5 mL, removing the supernatant and pelleting additional culture on top of the pellet. The amount of culture utilized depends on number of cells - too many cells will saturate the effectiveness of the protocol and lead to low yield and/or impure DNA. The cell volume should be at most ~ 30 µL).

3) Resuspend cells in 100 µL resuspension buffer (50 mM Tris-HCl, 10 mM EDTA, pH 8.0) and 5 µL (20 µg) 4 mg/mL RNaseA. This is best done by ~5 swipes on an eppendorf tray. Let stand for 2 min at RT.

4) Add 100 µL lysis buffer (200 mM NaOH, 1% SDS) and mix gently. Let stand for 2 min at RT.

5) Add 120 µL neutralization buffer (3 M KOAc, pH 5.5) and mix gently. Let stand for 3 min at RT.

6) Centrifuge at 11,000*g* for 1 min. Transfer supernatant to fresh tube.

7) Add 200 µL isopropanol to precipitate plasmid DNA, mix, and let stand for 1 min at RT.

1. Centrifuge at 11,000*g* for 1 min. Pipette off supernatant.
2. Wash DNA pellet by adding 100 µL 70% EtOH. Centrifuge at 11,000*g* for 1 min. Pipette off supernatant. Let air dry for about 10 min by inverting tube on a Kimwipe.

10) Resuspend in 30 µL sterile water to dissolve air-dried DNA. Store at -20°C.

11) Use 1-3 µL of plasmid solution for diagnostic digests. Measure concentration of a 1:100 dilution of DNA (in water) using 260 ≈ 20 mL/mg dsDNA [conc = (A/260)(dilution)]. An A260/A280 ratio of 1.8-2.0 is considered clean DNA.

**Boiling Miniprep** (clean, cuttable, sequenceable; no phenol, no tube changes)

1) Grow overnight cultures in LB + appropriate antibiotic(s).

2) Transfer 1.5 mL of culture into an eppendorf tube and centrifuge 30 s (~3000g). Remove supernatant. (More than 1.5 mL can be pelleted in a single eppendorf tube by pelleting 1.5 mL, removing the supernatant and pelleting additional culture on top of the pellet. The amount of culture utilized depends on number of cells - too many cells will saturate the effectiveness of the protocol and lead to low yield and/or impure DNA. The cell volume should be at most ~ 30 µL).

3) Resuspend cells in 300 µL STET (8% sucrose, 5% TX-100, 50 mM EDTA, 50 mM Tris, pH 8.0). This is best done by ~5 swipes on a eppendorf tray.

4) Add 20 µL 10 mg/mL lysozyme in 50 mM Tris, pH 8.0 (stored at -20 °C). Mix well and incubate at RT for 15 s to 10 min (5 min good average).

5) Place in boiling water bath for 2 min. Timing is important, so do not over- or undershoot.

6) Centrifuge 5 min (~6000g). Remove "snot" pellet with a sterile toothpick.

7) Add 325 µL isopropanol : 2.5 M NH4OAc (3 : 1) mixture to the supernatant. Centrifuge 5 min (~6000g).

1. Wash pellet with 100 µL 70% EtOH (add 70% EtOH and centrifuge 5 min). Dry in speed-vac 5 min (or leave tube upside down on bench for ~10 min).

9) Resuspend in 20 µL DI water. Add 0.5 µL RNase and heat at 65°C for 10 min (heat inactivates any DNases present).

10) Use 1-3 µL of plasmid solution for diagnostic digests. Measure concentration of a 1:100 dilution of DNA (in water) using 260 ≈ 20 mL/mg dsDNA. An A260/A280 ratio of 1.8-2.0 is considered clean DNA.

**Alkaline Lysis Miniprep**

1) Grow overnight cultures in LB or TB + appropriate antibiotics

2) Transfer 1-4 mL of culture into an eppendorf tube and centrifuge 30 s. More than 1 mL can be pelleted in a single eppendorf tube by pelleting 1 mL, removing the supernatant and pelleting additional culture on top of the pellet . (The amount of culture utilized depends on number of cells - too many cells will saturate the effectiveness of the protocol and lead to low yield and/or impure DNA. The cell volume should be at most ~ 50 µL).

3) Remove the supernatant from the pelleted cells (do not leave more than 20 µL of supernatant - it can interfere with lysis).

4) Fully resuspend cells in 200 µL lysis buffer: 2 mL 5% glucose

 2 mL 50 mM Na2EDTA

 5.75 mL H2O

 0.25 mL 1 M Tris, pH 8.0

 Incubate on ice 10-30 min (not more than 30 min).

5) Add 400 µL alkalic SDS (make fresh each time): 7 mL H2O

 2 mL 1 N NaOH

 1 mL 10% SDS

 Mix carefully, inverting tube until solution becomes clear.

6) Add 300 µL 3 M NaOAc, pH 4.8. Incubate on ice for 10 min.

7) Centrifuge at full speed for 5-10 min. Transfer supernatant to a new eppendorf tube and discard pellet (alternatively, remove "snot" pellet with a toothpick).

8) Add 600 µL isopropanol and incubate at -20°C for 5-10 min (all DNA and RNA should precipitate here).

9) Centrifuge 5-10 min and remove supernatant. Resuspend pellet in 400 µL 2.5 M NH4OAc (be careful, as pellet may stick to side of tube or pipet tip), and incubate at RT for 30 min.

10) Centrifuge for 5 min and transfer supernatant to a fresh tube (the pellet should be chromosomal DNA - discard).

11) Add 250 µL isopropanol. Incubate at -20°C for 10 min (overnight incubation results in MUCH greater yield).

1. Centrifuge for 10 min and remove supernatant (the pellet is plasmid DNA). Wash pellet with 100 µL 70% EtOH (pellet may not be visible). Dry in speed-vac 5 min (or leave tube upside down on bench for ~10 min).

13) Resuspend pellet in 20-40 µL DI water. Add 0.5 µL RNase and heat at 65°C for 10 min (heat inactivates any DNases present).

14) Use 1-3 µL of plasmid solution for diagnostic digests. Measure concentration of a 1:100 dilution of DNA (in water) using 260 ≈ 20 mL/mg dsDNA. An A260/A280 ratio of 1.8-2.0 is considered clean DNA.