Plasmid Mini-Preps using Qiagen solutions and No Columns

Marty Taylor – Boeke Lab. Last modified 12/2010. Based on Invitrogen Bacmid Isolation protocol and on Ed Davis's protocol from the Hughes lab. All spins at full speed in benchtop microcentrifuge at room temperature. <u>It is important to line the lids up so all point the same way – that way you know where the pellets are!</u>

1. Spin down 1.5/2.0 ml of overnight bacterial culture or any cell suspension 30s in a microcentrifuge tube. Remove as much supernatant as possible (vacuum aspirator helpful)
   a. Store glycerol stocks from leftover culture – I mix 666µL culture with 333µL 50% glycerol

2. Resuspend in 300/400µl of Qiagen's P1 buffer containing RNase A by vortexing (or for fewer samples, pipetting works well). A multi-sample vortex head or Turbomix is very useful.

3. Add 300/400µl of room temperature P2 (lysis) solution and mix by inversion. Incubate at room temperature for at least 5 minutes. The solution should change from opaque to almost clear. This incubation is critical for removal of RNA from the sample!

4. Add 300/400µl of P3 (neutralization) buffer and mix by inversion (**do not vortex**). Buffer N3 can be used in place of P3 in a pinch, but the resulting DNA will contain more RNA and may work poorly with large plasmids. **Optional:** incubate on ice 5-10 min.

5. Centrifuge 5 min. If using N3 instead of P3, centrifuge 10-15min (pellet is less firm in N3).

6. **Optional:** Phenol/chloroform/Iso-Amyl alcohol extraction – cleans up the DNA, but expensive and we have found it doesn't make a difference for digests or sequencing, so I don't do it.
   a. Transfer the supernatant to a new tube containing an equal volume of cold Ph/C/Iso-AA
   b. Spin 5min at full speed at 4°C, take the top (aqueous phase) and proceed to IPA precip

7. Transfer 700-800/1000µl supernatant into a new tube containing 800/1000µl isopropanol. Avoid transferring the white pellet. Mix by inversion and incubate on ice at least 5 min
   a. Pause point: can store at -20°C overnight or longer, but yield is not much changed

8. Centrifuge DNA precipitate 5 minutes. Discard supernatant; be careful – isopropanol pellets are only weakly adhered to the side of the tube and can be easily lost. Align the tubes before spinning!
   a. Note: extending centrifuge time can increase yield

9. Rinse DNA pellet with 500µl of 70% EtOH. Centrifuge 5 minutes and aspirate all supernatant
   a. Optional: repeat step 8.
   b. Optional: centrifuge pellet again 10 seconds and remove supernatant

10. Air dry pellet at room temperature for 5-10 minutes.

11. Resuspend in 50µl of 10mM Tris, pH 8.0. TE is fine too, if the EDTA doesn't interfere with your next steps. Use 0.5 µl in a 10 µl restriction enzyme digest – typical yield is around 500-1500ng/uL in 50uL with high copy plasmids when starting with 1.5mL culture.

Buffer P1 (store at 4°C after RNase A added)
    50 mM Tris-HCl pH 8.0 (6.06g/L)
    10 mM EDTA (3.72g/L disodium salt dihydrate)
    100 µg/ml RNaseA (Qiagen 19051 or 19101 or we can make it)
Buffer P2:
    200 mM NaOH (8 g/L)
    1% SDS (10g/L or 50mL 20% solution)
Buffer P3: 3.0 M potassium acetate pH 5.5 (294.4g KOAc, ~110mL glacial acetic acid per L)