Plasmid Minis

Procedure:

(1) Pick an overnight into 3-5ml media with the appropriate antibiotic selection.

(2) Transfer ~1.5mls worth of cells to a 1.5ml microfuge tube.

(3) Spin 2min (RT or 4^{o}C)

(4) Aspirate off supernatant.

(5) Quick spin to pull down the remaining spent liquid medium.

(6) Aspirate off remaining supernatant.

(7) Resuspend in 100μL lysozyme solution*.

(8) Place tube on ice (all reactions are performed on ice from here on out).

(9) Add 200μL 0.2N NaOH/1% SDS sol'n. Invert gently to mix, do not vortex or mix vigorously.

(10) Add 150μL 3M NaOAc (pH 4.0 - 4.5). Invert gently to mix, do not vortex or mix vigorously.

(11) Incubate on ice ~5min (or more).

(12) Spin in a microfuge at 4^{o}C for 15min (or more).

(13) Pull supernatant to a new tube; avoid the flakes & crud.

(14) Add 2 - 2.5 volumes EtOH (~1ml 95%). Thoroughly mix by inverting tube.

(15) Spin RT 2min.

(16) Decant s/n.

(17) Wash pellet w/ 1ml 70% EtOH.

(18) Spin RT 2min.

(19) Decant off EtOH washate.

(20) Dry pellet in speed vacuum 5-10min.

(21) Resuspend in 100μL (or any convenient volume) $T_{10}E_{0.1}$.

Sol'ns and stuff:

(1) Lysozyme sol'n (100ml)

(a) dH_2O	85.5ml	
(b) A/C		
(c) 50% Glucose	10ml	
(d) 1.0M Tris pH 8.0	2.5ml	[25mM]
(c) 0.5M EDTA	2.0ml	[10mM]

(2) 0.2N NaOH/1%SDS (10ml)

(a) 10N NaOH	200µL	[0.2N]
(b) 10% SDS	1.0ml	[1%]
(c) dH_2O	to 10ml	
(d) Store RT in plastic tube		

(3) 3M NaOAc pH ~ 4.0 - 4.5 (low pH is critical for 3M NaOAc used in minipreps because it must neutralizes the NaOH)

(a) NaOAc	40.81g
(b) Glacial acetic acid	~50ml
(c) pH to	4.0 - 4.5
(d) dH_2O to	100ml
(e) f, A/C	

(4) $T_{10}E_{0.1}$ (100ml)

(a) 1M Tris (pH 7.6)	1.0ml
(b) 0.5M EDTA	20µL
(c) dH_2O	99ml
(d) A/C	

Notes:

*Lysozyme sol'n used to call for lysozyme but eventually it was realized that the lysozyme was non-essential.

It is assumed that the stock sol'ns used to prepare buffers/sol'ns are sterile.

This procedure works well for a variety of vectors and host strains (even Agro). While the DNA is not clean enough to sequence, the quality is usually fairly high. Plasmid DNAs may be restriction digested and bands isolated from gels. For very clean DNA that can be used in *in vitro* transcription-translation reactions and/or for sequencing, use the EluQuick kit from S&S (which does not use RNase). After the DNA is resuspended, I spin it in the microfuge. Usually there is a white pellet, which I think is left over flakes & crud that was taken with the supernatant. It does not seem to interfere with most restriction digestions however.

Reference:

Birnboim and Doly (1979) A rapid alkaline procedure for screening recombinant plasmid DNA. Nuc. Acids Res. 7:1513-1523.