

Simple Plasmid DNA minipreps

Notes

- i) This is a very quick and dirty method, but eminently scalable and with very little hands-on time.
- ii) There is no RNase-treatment step. To remove RNA you can add 1 μl 10 mg ml^{-1} DNase-free RNase at step 5 and stand for 10 minutes before phenol/chloroform extraction followed by isopropanol precipitation OR (and this is what I do) simply add RNase to the restriction digest pre-mix. I'm assuming that's why you're doing minipreps in the first place. . .

Reagents

GTE:

1% glucose
10 mM EDTA
50 mM Tris pH 8.0

NaOH/SDS:

200 mM
1% SDS

KOAc (in 1 litre):

300 g potassium acetate
115 ml glacial acetic acid

TE:

1 mM EDTA
10 mM Tris pH 8.0

300 mM sodium acetate in TE

Ethanol

Method

1. Grow 2.5 ml overnight culture (shaking, 37°C), from a single colony, in 2xTY or similar rich media, plus appropriate antibiotic.
2. Pellet 1 – 2 ml culture, 30 seconds in microfuge.
3. Resuspend pellet (be as vigorous as you like) in 100 μl cold GTE and place on ice.
4. Add 300 μl NaOH/SDS and mix gently by inversion (3 - 4 times). Neutralize with 300 μl KOAc and mix thoroughly but gently by inverting \sim 4 times. If you are too vigorous at this point you will shear the genomic DNA and the prep will fail.
5. Microfuge at full speed, 10 minutes, RT. Take 600 μl supernatant into clean tube, avoiding any white floaty bits.
6. Add 450 μl propan-2-ol, mix well by inversion and stand at -20°C for 20 minutes.
7. Spin 10 minutes at full speed in microfuge. Remove supernatant and resuspend pellet in 400 μl 0.3 M sodium acetate in TE.
8. Add 1 ml ethanol, vortex, and precipitate at -20°C for 20 minutes.
9. Spin 10 minutes at full speed in microfuge, air-dry and resuspend in 50 – 100 μl TE.