Alkaline Lysis Plasmid DNA Minipreps

1. Pour 1.5 ml of each overnight culture into a labeled microfuge tube and cap.

2. Load centrifuge symmetrically using balance tubes if necessary. Centrifuge at maximum speed for 30s.

3. Pour out supernatant (should be clear) into a waste container. The cell pellet containing DNA remains in the tube.

4. Refill the tube with the remainder of the culture and repeat steps 1-3. You now have cells from ~3 mls of culture.

5. Add 100 μl GTE and resuspend the cell pellet by raking over 5x16 tube rack or vigorous vortexing.

6. Incubate 5 min at room temperature

7. Add 200 μl of alkali-SDS (fresh) and mix by gentle inversion the suspension will clear and turn viscous. As the SDS dissolves the cell membrane and denatures proteins chromosomal DNA is liberated and denatured at High pH.

8. Incubate 5 min on ice

9. Add 150 μl of Potassium Acetate solution and vortex to mix. The solution turns cloudyand even more viscous as the DNA renatures and S(Sodium)DS-Protein complexes precipitate as KDS complexes.

10. Incubate 5 min on ice

11. Spin down 5 min at room temperature

12. Transfer supernatant (~450 μl) to new tube

13. Add 0.9 ml cold ethanol to the supernatant (plasmid DNA will precipitate).

14. Spin down 5 min at room temperature.

16. Pour out supernatant into waste container. Invert the tube and dab onto a paper towel

15. Gently rinse the pellet with 70% ethanol. Pour out 70% ethanol and blot as before. Dry 5 min in a vacuum desicator or 20 min in air.

16. Resuspend in 50 μl TE.

17. Yield is approximately 3 μg for high copy number plasmids, 0.3 to 1μg for low copy number plasmids

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GTE	for 100 mls	for 200 mls
50 mM glucose	0.9 g (also called dextrose)	1.8 g
25 mM Tris, pH 8	2.5 ml 1 M stock	5 ml 1 M stock
10 mM EDTA	4 ml 0.25 M stock	4 ml 0.5 M stock

Alkali-SDS	for 10 mls
0.2 M NaOH	1 ml 2 M NaOH (2M NaOH is 4g /50 mls)
1% SDS	1 ml 10% SDS
	8 ml H_2O

Potassium Acetate Solution, 5 M, pH 4.8 (for 100mls)
Add 29.5 ml of glacial acetic acid (HOAc) to ~50 mls of H_2O
Adjust to pH 4.8 by addition of KOH pellets (~12 g)
Add H_2O to 100 ml

TE is 10mM Tris, 1mM EDTA pH 8.0