

Alkaline Lysis of Bacteria and Plasmid Prep

- 1) Pellet bacteria by spinning 10min at 5k at 4°C in big sorvall rotor.
- 2) Resuspend Pellet in 16ml of solution 1. Transfer to pop top polypropylene tube.

Solution 1

5 ml 20% Glucose
2.5 ml 1M Tris pH 8.0
2ml .5M EDTA pH 8.6
Add H₂O up to 100 ml

- 3) Add 400 µl of 200mg/ml lysozyme to each pellet. Incubate at RT for 5 min.
- 4) Add 32 ml of solution 2 to each pellet. Shake and leave on ice for 10 min.

Solution 2

2 ml 10M NaOH (0.2M final)
5 ml 20% SDS (1% final)

- 5) Add 24 ml of Solution 3. Shake and leave on ice for 10 min. If using MC1061P3 use 48ml

Solution 3

300 ml 5M Potassium Acetate
57.5 ml Glacial Acetic Acid
Add H₂O up to 500 ml (143 ml)

- 6) Spin pellets at 5k for 10 min at 4°C in 500ml bottles.
- 7) Remove supernatant and filter over cheesecloth.
- 8) Add .6 volumes of isopropanol (~42 ml/ pellet).
- 9) Spin 5k for 10 min.
- 10) Resuspend in 12ml 1xTE and add 12ml 5M LiCl
- 11) Spin 10k for 10min (10min @3k in Table top)
- 12) Remove Supernatant and add equal vol isopropanol.
- 13) Spin 10k for 10min or 3K for 10min in table top.
- 14) Resuspend pellet in 3.5ml TE and add 4.4g CsCl + 50µl EtBr and filter through .45µ (30ml TE /32g CsCl for Vti50)
- 15) Spin 4hr-O/N at 60k. (16+hrs @45k)
- 16) Repeat step 16
- 17) Pull band.
- 18) Extract with H₂O Sat'd butanol.
- 19) Add 1/10 vol. 3M NaOAc and 2-2.5 vol of 100%EtOH
- 20) EtOH ppt 2x and resuspend in 200-1000µl.