

Resuspend pellets in 100 ul of Solution I (Tris-EDTA-glucose, 50mM glucose, 10mM EDTA, 25mM . 1 Tris pH 8.0 – storage at 4°C) place on ice for 5 minutes. Add 150ul of ice-cold Solution III (for 100ml, 60ml of 5M KOAc, 11.5ml glacial Acetic Acid, 28.5ml H₂O) and mix by inverting the tube gently. Grow overnight cultures of E. coli (1.5–2.0 ml) in LB broth at 37°C using appropriate antibiotic selection. Harvest cells in microfuge tube by centrifuging for 5 minutes at 4,000 rpm, Discard media. Dry pellets (5 minutes in speedvac) and resuspend in 20–40ul TE + RNase A. Shaking for ~ 10 minutes was done to resuspend DNA pellets. Add 200 ul of freshly prepared Solution II (0.2N NaOH, 1%SDS). The solution mixture should be turbid, incubate on ice for 5 min. Chloroform will remove soluble proteins from .the preps. 2.3.4.5.6.7.8.9. 10. 11. 12. 13