WARNING NOTICE: The experiments described in these materials are potentially hazardous and require a high level of safety training, special facilities and equipment, and supervision by appropriate individuals. You bear the sole responsibility, liability, and risk for the implementation of such safety procedures and measures. MIT shall have no responsibility, liability, or risk for the content or implementation of any of the material presented. Legal Notices

GENOMIC DNA PREPARATION FROM *E. COLI* CELLS

Adapted from Current Protocols in Molecular Biology (2.4.1-2.4.2)

1. Spin 3 ml from an overnight culture of *E. coli* cells at maximum speed in a microcentrifuge fro 2 minutes; discard the supernatant
2. Resuspend the cell pellet in 567 µl TE (10 mM Tris, 1 mM EDTA, pH 8.0)
3. Add 30 µl 10% SDS and 3 µl 20 mg/ml proteinase K; mix thoroughly and incubate 1 hr at 37ºC
4. Add an equal volume of phenol:chloroform:isoamyl alcohol (25:24:1); mix well by shaking up and down vigorously (do not vortex); spin in microcentrifuge at maximum speed for 10 minutes
5. Transfer the aqueous top layer to a fresh microcentrifuge tube with a Pasteur pipette; add an equal volume of phenol:chloroform:isoamyl alcohol; mix well and microcentrifuge for 10 minutes
6. Transfer the aqueous top layer to a fresh microcentrifuge tube with a Pasteur pipette; add an equal volume of chloroform:isoamyl alcohol (24:1); mix well; centrifuge for 5 minutes
7. Transfer the aqueous top layer to a fresh microcentrifuge tube with a Pasteur pipette; add 1/10 volume of 3M sodium acetate pH 5.3; mix
8. Add 3 volumes of cold 95-100% ethanol; mix
9. Centrifuge at maximum speed for 15 minutes
10. Wash the DNA pellet with cold 70% ethanol; discard the supernatant
11. Dry the DNA pellet completely and resuspend in 100 µl TE

©P. LESSARD 2003, ALL RIGHTS RESERVED