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 *CORYNEBACTERIUM* AND *RHODOCOCCUS*, MINI-PROTOCOL

1. Spin overnight culture (grown in 10ml LB) for 5 min at 6000 rpm in GSA rotor or equivalent
2. Pour off supernatant and freeze cell pellet at -20°c for 30-60 min. (or overnight at -70°C)
3. Resuspend cell pellet in 250µl fresh 10mg / ml lysozyme in TE and transfer to microcentrifuge tube
4. Add 20 µl mutanolysin (1 mg / ml)
5. Incubate at 37°c for 1-2 hr while gently shaking
6. Add 50 µl 0.5 M EDTA, 50µl 10% SDS, 50 µl 5 M NaCl; Mix gently
7. Add 10 µl fresh 20mg / ml proteinase K (or Sigma cat. no. P5568) and incubate at 37°c for 60 min.
8. Add 50 µl sodium perchlorate solution (1 g / ml) to the cell slurry; mix gently
9. Extract with one volume of phenol:chloroform:isoamyl alcohol (25:24:1); mix well; centrifuge 5 min in microcentrifuge
10. Extract aqueous phase (beware phase inversion) with 0.5 volumes of chloroform:isoamyl alcohol (24:1); mix well; centrifuge 5 min. in microcentrifuge
11. To aqueous phase add 1 volume isopropanol; microcentrifuge sample for 15 min;
12. Wash pellet; dry, resuspend DNA in 200 µl TE; may leave overnight at 4°C

For routine use, you may stop here; for higher quality DNA, proceed to step 13.

13. Add 1 µl RNase (0.5 mg / ml) and incubate at 37°c for 30 min.
14. Add 0.5 volumes 7.5M ammonium acetate
15. Extract DNA / ammonium acetate solution with one volume phenol:chloroform:isoamyl alcohol; centrifuge 5 min. in microcentrifuge
16. Extract aqueous phase with 0.5 volumes chloroform:isoamyl alcohol; centrifuge 5 min. in microcentrifuge
17. To aqueous phase add 2 volumes ethanol; microcentrifuge sample for 15 min;
18. Wash DNA in 70% ethanol, dry and resuspend in 200 µl TE.