# *MYXOCOCCUS* CHROMOSOMAL DNA PREP

*Adapted from existing protocols by Vinh Pham.*
*Last modified: June 2, 1999*

MATERIALS:

**STE** (25% sucrose, 10mM Tris pH 8, 1mM EDTA)
**Rapid Lysis Mix** (5% SDS, 0.5M Tris pH 7.6, 125mM EDTA)
**Tris-buffered Phenol** DNA will enter organic phase if pH of phenol is below 7.8-8.0. Oxidation of phenol can be minimized by the addition of 8-OH-quinoline (0.1%).
**Chloroform:isoamyl alcohol (24:1)** The isoamyl alcohol minimizes foaming.
**Tris-EDTA** (10mM Tris pH 8.0, 1mM EDTA**)**
**Ethanol** (70% and 95%)
**5M Potassium Acetate** (3M potassium, 5M acetate; to make 100ml, mix 11.5ml glacial acetic acid, 28.5ml $dH_2O$, 60ml 5M KOAc)

PROCEDURE:

1. Grow *Myxococcus* cells in CTT or CTTYE medium at 33ºC.
2. Spin down 2-5ml of these cells at 10K rpm for 5 minutes. More cells means more gDNA, but also more cell debris.
3. Resuspend in 400µl STE.
4. Freeze at -20ºC for at least 30'.
5. Thaw, then add 80µl RLM.
6. Mix by inversion.
7. Heat at 70 ºC for 30'.
8. Add 100µl 5M potassium acetate, vortex 30".
9. Allow to cool at RT for 10-15', vortexing every 5'.
10. Spin for 10' at 14K rpm.
11. Transfer supernatant into new tube (the DNA is in the SN).
12. Add 750µl Tris-saturated (or, TE- or buffer-saturated) phenol to tube.
13. Invert to mix and spin 5' at 14K rpm.
14. Transfer aqueous (BOTTOM) layer to new tube (test with a drop of Tris or TE to distinguish between the organic and aqueous phases). Avoid transferring cell debris.
15. Add 1ml chloroform/isoamyl alcohol.
16. Invert to mix and spin 5' at 14K rpm.
17. Remove aqueous layer to new tube.
18. Add 95% or 100% EtOH to fill tube.
19. Invert 20-50 times. The DNA should become visible as white, stringy globs.
20. Spin 30" at 14K rpm.
21. Pour off EtOH, wash with 1ml 70% EtOH, then with 1ml 95% EtOH.
20. Air dry (DO NOT use SPEEDVAC) until pellet turns clear.

22. Add 20-100 µl TE or 10mM Tris, pH 8.5 to resuspend the DNA (heat in 37ºC water bath to help resuspend DNA).