MYXOCOCCUS CHROMOSOMAL DNA PREP
Adapted from existing protocols by Vinh Pham.
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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%).
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 dH2O, 60ml 5M KOAc)

PROCEDURE:

1. Grow Myxococcus cells in CTT or CTTYE medium at 33°C.
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 -20°C for at least 30’.
5. Thaw, then add 80μl RLM.
7. Heat at 70°C 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.
22. Air dry (DO NOT use SPEEDVAC) until pellet turns clear.
22. Add 20-100μl TE or 10mM Tris, pH 8.5 to resuspend the DNA (heat in 37°C water bath to help resuspend DNA).