PRONASE METHOD FOR MAKING DNA FORM C.R.
from Erickson, J., et al. Molecular biology of C. Chp. 10

1. Grow cells to stationary phase in TAP.

2. Pellet at 5K, 2-5 min, 4° C.

3. Resuspend in 20 ml. TAP medium. Transfer to a 30 ml. Corex tube or a Sarstedt tube, and centrifuge at 3K, 5 min. 4° C.

4. Gently resuspend pellet in 8 ml. of 4° C buffer A and transfer the suspension to a 50 ml. Erlenmeyer flask.
   Buffer A:
   0.1 M NaCl
   50 mM EDTA
   20 mM Tris-HCL, pH=8.0

5. Add 0.5 ml. of pronase solution, swirl to mix.
   pronase solution:
   10 mg/ml in 0.01 sodium citrate [pH=5.0], predigested for 2 hrs. at 37°C and stored frozen.

6. Add 0.5 ml of 20% SDS to cells (2.0 ml for wild type), and place in 50° C water bath.

7. After 45 minutes of incubation, add 0.5 ml pronase. Repeat 45 minutes later. (Note: After 2-2.5 hrs. incubation, cells will turn brown, then proceed to phenol extraction)

8. Cool cells on ice and add approx. 20 ml phenol [saturated overnight with 0.1 M sodium borate]. Leave in ice 20 min. with occasional swirling.

9. Remove top pink aqueous phase and purify by further CsCl gradient centrifugation.

10. Precipitate DNA by adding 2 vols. -20° C absolute ethanol.

11. Spool DNA into another tube and rinse with cold 70% ethanol. Note: If DNA is visualized with Hoechst's dye, do not centrifuge after ethanol addition.

12. Vacuum dry briefly, until sides of tube are dry and add 10mM Tris-HCL [pH=8.0] until pellet is covered. Leave at 4° C overnite until DNA is dissolved.