Mini-Chromosomal (Total) DNA preparation from *Sulfolobus*. (Modified from Schleper)

1. Grow up at least 2 ml culture of *Sulfolobus* to late log phase (or stationary).

2. Spin 1.5 ml in microcentrifuge tube for 30 seconds at top speed in microcentrifuge.

3. Discard supernatant (or keep to check for viruses).

4. Resuspend pellet in 250 μl TEN (see below).

5. Add 250 μl TENST (see below) mix by inverting tube.

6. Incubate for 30 minutes at room temperature.

7. (in the hood and wearing gloves!) add 500 μl phenol/chloroform/isoamyl alcohol and mix by vortexing.

8. Spin for 2 minutes at maximum rpm in micro-centrifuge.

9. Remove the upper (aqueous layer) with a micropipette (take a small amount of the interface) and put in a new microcentrifuge tube.

10. Repeat steps 7, 8 and 9.

11. If there is still a lot of interface repeat steps 7, 8 and 9, if not proceed to step 12.

12. Remove 450 μl (or less) of the aqueous (upper) phase and place in a new microcentrifuge tube.

13. Add 50 μl of 3M sodium acetate (NaOAc) and 1 ml ethanol (EtOH).

14. Incubate for 15 minutes on ice.

15. Spin for 15 minutes at maximum rpm in micro-centrifuge, there should be an obvious pellet.

16. Remove and discard supernatant.

17. Add 500 μl 70% ethanol to pellet, mix briefly by pipetting up and down.

18. Spin for 2 minutes at maximum rpm in micro-centrifuge

19. Air dry the pellet for ca 15 minutes at room temperature. (or 2-3 minutes in Speed-Vac).

20. Dissolve in 15 μl TE (+ RNAse if you are not looking for RNA – elements).

21. Cut 5 μl with a restriction endonuclease (*Eco*RI) and analyze by agarose gel electrophoresis.

TEN: 10 mM tris/HCl pH 8.0, 1 mM EDTA, 150 mM NaCl
TENST: TEN containing 0.12% triton X-100 and 1.6% sarcosine.
Ken Stedman 6 November 2002