***S.solfataricus* Electroporation (from C. Schleper), modified by Stedman.**

Preparation: Previous day: inoculate 50ml Yeast-Sucrose (Y.-S.) mediumwith 50μl *S.solfataricus* stock culture. Incubate overnight at 80°C. Put 100ml sterile 20mM sucrose per culture at 4°C.

Immediately before electroporation: Preheat >1ml medium per electroporation to 80°C. Put 1 x 100ml growth flask containing 50ml Y.-S. medium per electroporation at 80°C.

Electroporation Protocol:

1. Measure OD at 600nm. If over 0.2 dilute to at most 0.1 with fresh medium and let grow.

2. At OD 600nm = 0.2 cool the culture on ice for at least 15 minutes . Pre-cool centrifuge (10°C max.).

3. Spin culture for 10 minutes at 3000 rpm. (IEC- 981 swinging bucket rotor (ca 5000 x g)). Carefully discard supernatant.

4. Resuspend pellet in 50ml cold 20mM sucrose with pipette (DO NOT VORTEX!).
   (add ca. 10ml sucrose and pipette up and down gently with P1000 micropipette.)

5. Repeat Step 3. During the centrifugation steps set up microdialysis of DNA (see below).

6. Resuspend pellet in 25ml cold 20mM sucrose with pipette (DO NOT VORTEX!).

7. Repeat Step 3.

8. Resuspend pellet in 10ml cold 20mM sucrose with pipette (DO NOT VORTEX!).

9. Repeat Step 3.

10. Resuspend pellet in 1ml cold 20mM sucrose with pipette (DO NOT VORTEX!).

11. Repeat Step 3.

12. Resuspend pellet in 400μl cold 20mM sucrose with pipette (DO NOT VORTEX!).

13. Place 1μl DNA solution (1:10 diluted with distilled water, or 10μl microdialysed) in each electroporations cuvette (0.1 cm). DNA amounts generally from 100ng to 1ug.

14. Place 50μl cell-suspension in each electroporations cuvette (0.1 cm).

15. Electroporate: 1.5 kV, 400 Ω, 25 μF. (The time constant should be about 9.0 ms)
   (Bio-Rad Gene pulser II with Pulse controller)

16. Immediately add 1 ml of hot (80°C) medium.

17. Leave 1 hour at 80°C.

18. Transfer 1 ml to the 50ml of preheated medium.

19. Let grow for 2 days at 80°C.

20. Test for virus-production/UV induce

(Generally test by spot on lawn procedure, but growth inhibition should be observable (relative to the negative control (no DNA)).

KMS 13.11.02

Microdialysis for electroporation. (modified from Advanced Bacterial Genetics Course, Cold Spring Harbor, Stanley Maloy, Valley Stewart, Ronald Taylor):

1. Dilute TE approximately 1:1000 with distilled water in a beaker.

2. Float a Millipore type VS membrane (or fragment thereof) on the surface of the diluted TE shiny side up.

3. Leave for 2 minutes.

4. Carefully spot 1-10 μl of DNA containing solution on the membrane with a micro-pipetter (can do multiple spots but make sure that membrane is marked, often by cutting one corner).

5. Leave for 20 minutes (more is possible, but the DNA solution may gain volume).

6. Remove DNA solution (set micropipettor to a higher volume than the original volume of the spot and carefully remove the liquid from the membrane.