

Sulfolobus lawns: / Halo assay

Need: Growing culture for lawn (usually *S. solfataricus* P1 or P2).

1. Turn on dry bath incubator (502) to 80°C and fill up as many of the large holes with water as you will have plates. Wait until incubator reaches 80°C.
2. Get as many Sulfolobus Y/S plates as you will need from 4C and warm to room temperature. Label the plates on the side with your initials, the date and the culture to be used for the lawn.
3. Weigh approximately 0.02g of Gelrite into a small beaker.
4. Add 10ml of Y/S medium to the Gelrite and boil with stirring (hot plate in 502).
5. Put plates in 80°C incubator (autoclave room)
6. Transfer 0.5 ml of lawn culture (OD 600 = 0.2 to 0.3) to 15 ml conical tube (orange cap) and place in 80°C dry bath incubator.
7. When Gelrite boils and dissolves, move beaker to dry bath incubator.
8. Get plates from 80°C incubator and place on dry bath incubator.
9. Add 3 ml of Gelrite to each tube of culture, swirl and pour on plate.
10. Remove the plates from the dry bath incubator and place on the benchtop until they are solid (When the rest of the Gelrite in the beaker solidifies is a good indication that the plates are solid.).
11. Spot 1-2 μ l of the culture/supernatant that you want to test on this lawn. Use 1 μ l of 1% Triton X-100 as a positive control for plaque formation.
12. Incubate the plates at 80C for 2+ days.
13. Observe halos.

K.M.S. 17 April 2003