

Transmission Electron Microscopy Sulfolobus Virus Screening protocol:
Parafilm technique

1. Get as many carbon-formvar TEM grids as you have samples to check.
 - a. Generally 300 mesh copper from Ted Pella or EM-sciences.
 - b. Can be plasma cleaned, but this is not necessary.
2. Cut a piece of Parafilm big enough to put all of your samples on.
 - a. I like about 3 x 3 cm per sample.
3. Spot 5 μ l of your sample (see below) on the parafilm
4. Spot 5 μ l of 2% uranyl acetate (wt/vol in water) on the parafilm about 1cm above your sample.
5. Carefully place grid on top of sample (carbon side down) with forceps.
6. Start timer
7. Lift the grid with the drop of sample adhered to it off the Parafilm
8. At 2 minutes hold a small piece of filter paper to the side of the grid perpendicular to the grid. This will wick off the liquid. Do not wick completely dry, leave a thin film of liquid on the grid.
9. Place the grid on the drop of uranyl acetate
10. Wait 15-30 seconds to stain.
11. Hold a small piece of filter paper to the side of the grid perpendicular to the grid. This will wick off the liquid.
 - a. Be sure to discard this filter paper in the uranyl acetate waste.
12. Air dry the grid.
 - a. 5-10 minutes is generally more than enough.
13. Examine the grid in the TEM.
 - a. Generally 15-20,000 x magnification is good for viruses.
 - b. It is often useful to find a cell (if your sample is a culture) or flagellum to focus on before screening for viruses.