



Phagehunting Program

EM Grid Preparation

1. Cover the lab counter with the plastic faced paper to create a clean area.
2. Using the pointed forceps, remove a fresh (unused) grid from the grid box of unused grids. Handle the grids by the edges with forceps only.
3. Place the edge of the grid beneath the parafilm in the Petri dish used for cleaning grids. Close the front (gray) valve.
4. Turn the valve to connect to pump (Figure 1). Pump out the vacuum desiccating chamber.
5. Using the vacuum tester, clean the grids (blasting the grid to remove dirt and oil).
NOTE: Touch the tester to the metal piece at the top for ~ 45 seconds. You should see a purple glow (ionized gas) above the Petri dish.
6. **Bleed vacuum before turning it off.** (Figure 2)
NOTE: This is to prevent backwash of oil into the pump.
7. Slow vent the vacuum to open (gray valve).
8. Remove the Petri dish containing the now cleaned grids.
9. With forceps, remove one grid.
NOTE: To keep the forceps closed, push the rubber ring down toward the bottom.
10. Lay the closed forceps holding the grid on top of the Petri dish.
11. Using a Pasteur pipette, spot the phage onto the grid. Use your thumb pressure on the back of the pipette to expel the drop.
12. Let stand ~ 1-2 minutes (depending on phage titer).
NOTE: Too much phage will burst the frame on the grid.
13. Using the pipette, remove the phage spot by capillary action.
14. Do not let the grid dry out.
15. Wash the grid by spotting with water. (6-7 times) To remove the water, turn the forceps on its side and gently tap on the Petri dish.
16. Using the Pasteur pipette, add a spot of stain. Let stand ~1 minute.
17. Remove the stain by knocking the drop off then wicking it off each side with Whatman paper. Be sure to wick, touching only around the edges to avoid contamination with paper fibers.

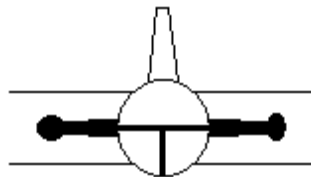


Figure 1 - Valve connected to pump

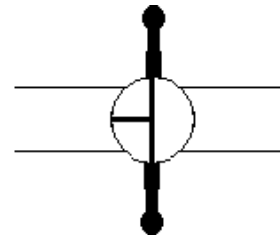


Figure 2: Bleed pump