



Phagehunting Program

Mycobacteriophage Protein Gel Electrophoresis

1. Transfer 50 μ l dialyzed phage to a clean eppendorf tube.
2. Spin at 14K RPM for 4 minutes to get rid of dust and other debris.
3. Transfer the supernatant to a fresh tube.
4. To the new tube with the supernatant, add 75 μ l distilled water and vortex.
5. Freeze/thaw on dry ice 3 times, vortexing in between.
6. Incubate at 37°C for 4 minutes.
7. Add 2 μ l (20 units) of DNA I.
8. Incubate at 37°C for 1 hour.
9. Add 25 μ l 4x SDS PAGE sample buffer.
10. Boil for three minutes.
11. Place on ice.
12. When the sample is cool, run the sample on a polyacrylamide gel, loading 5-20 μ l per lane, filling blank lanes with 20ul 1x sample buffer.

To purify proteins further, instead of step 9 above, continue here.

9. Add 20% TCA to 1:1 to the sample to get 10% TCA (total volume ~125ul). Vortex and incubate on ice for 5 minutes.
10. Centrifuge at 14K RPM for 5 minutes.
11. Wash with 1 ml ice cold acetone. Incubate for 2 minutes at room temperature.
12. Centrifuge and pour off the supernatant.
13. Repeat steps 11 and 12 twice more.
14. Air dry the pellet.

Note: For 1-D gels, resuspend the phage protein pellet in 50 μ l 4x SDS page sample buffer. Boil this for 3-5 minutes. Store in the freezer. For 2-D gel samples, air dry and store in the fridge as pellets.

Running the Gel

1. Obtain a precast polyacrylamide gel and remove it from its pouch. Remove the comb and rinse the wells with distilled water or running buffer.
2. Cut along the dotted line at the bottom of the gel cassette with a razor blade. Pull the clear tape at the bottom of the cassette to expose the bottom edge of the gel.
3. Place the precast gel into the electrode assembly with the short plate facing inward. Since only one gel will be run, and there are two places to run gels in the apparatus we're using, use the mini cell buffer dam for the side that has no gel.

4. Slide the gel cassette and electrode assembly into the clamping frame and press down on the electrode assembly while closing the two cam levers of the clamping frame. Now, lower this into the mini gel tank.
5. Fill the inner chamber with ~125 ml running buffer. The buffer level should be between the tops of the taller and shorter glass plates of the gel cassettes.
6. Add ~200ml running buffer to the mini-gel tank (lower buffer chamber).
7. Make sure the wells are filled with buffer and load 5-20 μ l per lane using flat tips, filling any blank lanes with 1x sample buffer. Be careful not to puncture the gel with the pipette tip.
8. Run the protein gel at 200V until the dye runs to the bottom of the gel.
9. Turn off the power supply, remove the tank lid, lift out the inner chamber assembly and pour the running buffer into the sink.
10. Open the cams of the of the clamping frame and pull the electrode assembly out. Remove the gel cassettes.
11. Remove the gels from the two plates, and transfer it to a small Tupperware container.
12. In the Tupperware container, add gel stain to cover the gel. Let this sit for ~10 minutes. Pour off the stain.
13. Rinse off all electrophoresis apparatuses with ddH₂O and set on a paper towel to dry.
14. Add destain to the container and pour it off. Add destain again to cover the gel and put a kimwipe in the container. Set on the rocker near the geldoc. Change the Kimwipe or the destain if needed. This may take up to a day. Photograph the gel and save the result in the geldoc computer folder and in the sever Phage Folder. Carefully label your photograph for your notebook.