Laboratory for Environmental Pathogens Research Department of Environmental Sciences University of Toledo

Polyvinylpyrrolidone (PVPP) cleanup of DNA samples

<u>Materials</u>

10% PVPP solution (sterile) Microcentrifuge tubes (sterile)

For each sample: Two-1.5 ml tubes

- Wear gloves throughout the entire protocol.
- Do not cross-contaminate your samples or the solutions. Be aware of your pipette tip.
- Work clean, either on fresh blue bench paper, in the hood, or on a freshly ethanol treated bench top.
- Perform all centrifugations with the hinge of the tube pointing "up".
- Do not use a vortex at any point in this protocol unless specified.

The protocol in brief

You will perform a cleanup of DNA by centrifugation of the sample through a column containing PVPP.

A. PVPP cleanup

1. Add 300 ml of sterile 10% PVPP solution to an empty spin column. Place the spin column in a sterile 1.5 ml microcentrifuge tube.



Empty spin columns are convenient for cleaning DNA with PVPP or any customized matrix.

- 2. Centrifuge at 14,000 x g for one minute. Empty the catch tube and repeat the centrifugation to dry the PVPP matrix. Place the spin column in a sterile 1.5 ml tube.
- 3. Increase the nucleic acid solution volume to at least 100 μ l by adding DNase/RNase-free water. Add the entire volume to the spin column and centrifuge for one minute at 14,000 x g.
- 4. Repeat step 3 if necessary.
- 5. If the resulting DNA concentration is too low for your application, then reprecipitate with isopropanol as described above and suspend the resulting pellet in a smaller volume of water.

Store your nucleic acid samples in the freezer (-20 °C), properly labeled. Do not assume that you will remember the extraction date, sample characteristics, etc. Write all of this information down in your lab book and mark your sample tubes accordingly.

Further reading

Menking, D.E., P.A. Emanuel, J.J. Valdes, and S.K. Kracke. 1999. Rapid cleanup of bacterial DNA from field samples. Resources Conserv. Recyc. 27:179-186.