Water Quality Testing
PCR-based Testing for Water Bacterial Contaminants

Part I. Isolation of DNA

Isolation of Bacteria From Water Samples

1. Label a tube containing the contaminated water sample with your initials.
2. After spinning the tube in a microcentrifuge at maximum speed for 15 minutes, remove the supernatant and place in a separate tube.
3. Add back 175 µl supernatant to the pellet and resuspend by vortexing.

Isolation of Bacterial DNA for PCR

1. Mix the tube containing the lysis solution (buffer, chelating agent and proteinase K) by inverting several times. Continue to mix with a pipet by pipeting up and down several times.
2. Rapidly add 50 µl of the lysis solution to the tube containing the supernatant. Mix well by inverting several times or by vortexing.
3. Heat the sample at 56 ^{o}C for 30 minutes. This will digest the bacterial cell wall and release the DNA.
4. Heat the sample in a boiling water bath for 10 minutes to denature and destroy the activity of the Proteinase K.
5. Allow the sample to cool to room temperature for 2 minutes.
6. Spin the tube at maximum speed in the microfuge for 5 minutes.
7. Transfer the supernatant to a clean tube and place the tube on ice. Be careful to not transfer the chelex to the PCR tube as it will inhibit the PCR process.
8. Dilute the DNA 1:100 with distilled water.

Part II. DNA Amplification

1. Label the tube containing the PCR reaction pellet with your initials and tap the tube to make sure the pellet is at the bottom of the tube.
2. Add 20.0 µl of primer solution and 5.0 µl of bacterial DNA from water sample to the tube.
3. Briefly spin in the microcentrifuge to collect the entire sample at the bottom of the tube. The PCR reaction pellet should be completely dissolved.
4. Make a negative control by adding 20.0 µl of primer solution and 5.0 µl of sterile water. Make a positive control by adding 20.0 µl of primer solution and 5.0 µl of *E. coli* DNA. Briefly spin in the microcentrifuge to collect the entire sample at the bottom of the tube. The PCR reaction pellet should be completely dissolved.
5. Place the water samples and control tubes in the thermal cycler and amplify using the cycling program on the next page:

Initial Denaturation: 95^{o} for 5 min
30 cycles @ 94^{o}C for 45 sec 50^{o}C for 45 sec 72^{o}C for 90 sec
Final Extension : 72^{o}C for 7 min

6. After the PCR has been completed, add 5 µl of 10x Gel Loading Solution to the sample and analyze by gel electrophoresis.

Part III. Gel Electrophoresis

1. Prepare a 1% agarose gel (0.25 g agarose, 0.5 mL 50X buffer, 24.5 mL distilled water). Have a water bath at 50 ^{o}C ready for heating tubes containing DNA fragments before gel loading.
2. Heat the 200 bp DNA ladder and PCR sample for 2 min at 50 ^{o}C. Allow the samples to cool for a few minutes.
3. Load the entire 25 µl sample from each tube onto the gel using the following sequence:

Lane	
1	200 bp DNA Ladder
2	*E. coli* positive control
3	Negative control
4	Water Sample 1
5	Water Sample 2
6	Water Sample 3

4. Run the gel at 125 volts. Allow the tracking dye to migrate about 4.5 cm from the wells to allow for adequate separation of the DNA bands. End the electrophoresis before the tracking dye moves off the end of the gel!
5. Remove the gel from the bed for staining with InstaStain ethidium bromide.
6. Wear gloves when doing anything with the ethidium bromide. Remove the clear plastic protective sheet on the stain card and place the unprinted side of the EtBr card on the gel. Firmly run your fingers over the entire surface to remove any air bubbles and make sure there is good contact between the card and the gel. Place a small beaker on top of the sheet and allow the gel to stain for 10-15 min.
7. Remove the card, and view the gel on a uv transiluminator for viewing (we will use one upstairs in the bio lab).