PCR Protocol for Environmental Bacterial DNA

Before class:
(1) Instructor adds to each master tube of Gibco Supermix (solution containing Taq polymerase, salts, magnesium chloride, and dNTPs): 2.5µl of 100µM primer 533F and 2.5µl of 100µM primer 1492R.
(2) Each master tube is divided in two, for two lab groups.

Thursday class

1. For each DNA sample: Aliquot 45µl of Supermix (containing primers) into a PCR-compatible tube.
2. Add 5µl of bacterial DNA sample to the tube.
3. Run PCR thermocycler under the following conditions:

    94°C for 2 minutes

    94°C for 30 s, 55°C for 30 s, 72°C for 60 s

    30 cycles

    72°C for 7 minutes

    4°C hold

5. Store samples in freezer.

Monday or Tuesday before next class

The result of the PCR amplification is tested by running a small portion on an electrophoretic gel.

1. Pour 100 ml 1X TAE Buffer with agarose and set aside.
2. Fill gel box up to gel surface plate with 1X TAE.
3. Microwave TAE/agarose until solution is clear. Let it cool to the touch.
4. Add 5 µl Ethidium Bromide to solution and pour into container. Let sit for 45 minutes or until gel is opaque. Cover gel with buffer after gel is solidified.
5. Take 5 µl of PCR product and add 1 µl of dye for run, then insert DNA and dye into gel wells. Add 6µl of 1kb DNA ladder to an end well for comparison.
6. Run gel at 100V until dye is halfway/ three-fourths down the gel. Observe on transilluminator (UV) with goggles. The expected size of the PCR amplification product between the two primers within the16S rRNA gene sequence is 1 kb.
7. Obtain digital image. Keep on file, and also provide digital copy to your instructor. This will be used to estimate the amount of product in each sample, when we send the DNA to OSU for sequencing.