

Protocol: Polymerase Chain Reaction

In this laboratory, we will amplify a region of the 16s rDNA gene (based on the *E. coli* 16s rDNA sequence) using Polymerase Chain Reaction (PCR). The DNA will be sequenced and we will then compare the results obtained with the public databases (NCBI and RDP) and determine the identity of the unknown bacteria.

1. Each student will obtain one liquid culture of bacteria. Note the code number. Also note the characteristics of the liquid culture such as color or consistency. Find the plate associated with the code number and also describe the characteristics of the colony on the plate.
2. Every unknown culture should be treated as though it were a human pathogen. Wear gloves while handling the bacteria. Be careful not to contaminate anything, especially yourself.
3. Carefully resuspend the culture in the test tube. Remove 1 mL of culture. Pellet in the microfuge for 2 minutes at 10,000 x g. Carefully draw off the medium using a P200. Some pellets are not firm and are easily disturbed.
4. Follow the **Invitrogen protocol** for purification of genomic DNA from bacteria.
5. Set up PCR reactions. Each student will add 8 uL of dH₂O and 2 uL of the DNA supernatant to the small (0.5 mL) PCR tube. You should have a total of 10 uL in the tube. Write your initials on the TOP of the tube.
6. The instructor will prepare Master Mix plus primers, as shown in the table below. The Master Mix contains all the common components for a set of reactions. It improves consistency among the reactions and reduces pipetting error.
7. We will set up an "assembly line" to add Master Mix and mineral oil before putting the tubes into the PCR machine. Add 40 uL of Master Mix plus the 10 uL already added to the PCR tube = 50 uL final reaction volume.

MASTER MIX

Reactant	per Reaction	x (number of students)+ 1 extra =
Taq Polymerase 5 U/ul	0.1 uL	_____ uL
PCR buffer 10x	5 uL	_____ uL
MgCl ₂ 1.5 mM	3 uL	_____ uL
each NTP 10 mM	1 uL	_____ uL
forward primer 10 mM	1 uL (1.0 mM)	_____ uL
reverse primer 10 mM	1 uL (1.0 mM)	_____ uL
dH ₂ O	15.4 uL	_____ uL
TOTAL	40 uL	_____ uL

PCR Parameters:

Initial Denaturation: 95°C - 3 min

30 cycles of:
94°C - 1 min
55°C - 1 min
72°C - 1 min

Linked to: 72°C - 10 min

Linked to: 5°C - indefinitely

Primer A - 28 nts - coding strand (310 ~340)	Primer B - 26 nts - non-coding strand (770~740)
5'CGGCCAGACTCCTACGGGAGGCAGCA-3'	5'-GCGTGGACTACCAGGGTATCTAATCC-3'

Questions to consider:

1. What features of 16s rDNA make it suitable for phylogenetic analysis?
2. Describe the process of PCR. What happens during one PCR cycle?

Adapted from:

"Identification of bacteria using two degenerate 16s rDNA sequencing primers" by Boye, et al., the "Microbiology Laboratory Manual" by J. D. Newman, Lycoming College (srv2.lycoming.edu/~newman), and Dr Dag Harmstad, University of Muenster, Germany (personal communication).