

# Molecular Epidemiology: DNA Fingerprinting

## Objectives

1. Define RFLP and PCR.
2. Perform agarose gel electrophoresis.
3. Identify your unknown.

## Background

Molecular epidemiology is used to identify strains of pathogens and use this information to track infectious disease. In DNA fingerprinting, DNA isolated from patients or environmental samples is compared to DNA of known organisms or other isolates. The isolated DNA is amplified (many copies of it make) by a technique called **polymerase chain reaction (PCR)**.

Starting with just one gene-sized piece of DNA, PCR can be used to make literally billions of copies in only a few hours (Figure 1). In the PCR technique, a solution containing the piece of DNA to be amplified is first heated to 98°C in a test tube to separate the two strands of DNA, which will serve as the initial templates for DNA synthesis. To this DNA is added a supply of the four nucleotides (for assembly into new DNA) and the enzyme for catalyzing the synthesis, DNA polymerase. Short pieces of nucleic acid called *oligonucleotide primers* are also added to help start the reaction. Then, during a period of incubation at 60°C, the primers hybridize to the ends of the fragments to be amplified, and the polymerase synthesizes new complementary strands. After each cycle of synthesis, the DNA is heated to convert all the new DNA into single strands. Each newly synthesized DNA strand serves in turn as template for more new DNA. As a result, the process proceeds exponentially. PCR is made possible by the use of DNA polymerase taken from a thermophilic bacterium such as *Thermus aquaticus*; the enzyme from such organisms can survive the heating phase without being destroyed. Thirty cycles will increase the amount of target DNA by more than a billion times.

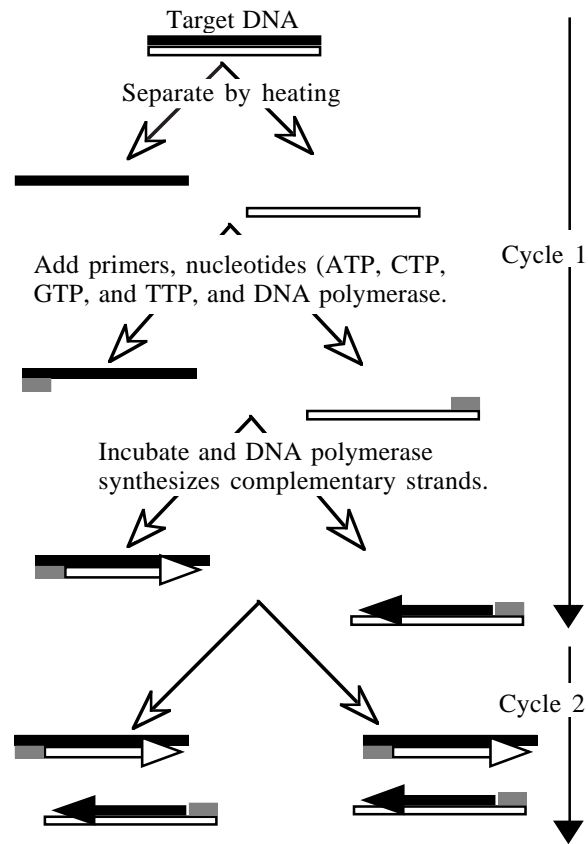


Figure 1. Polymerase chain reaction.

Amplified DNA is then digested by restriction endonucleases. **Restriction endonucleases** recognize specific short nucleotide sequences in double-stranded DNA and cleave both strands of the molecules (Table 1). Restriction endonucleases are bacterial enzymes that destroy unfamiliar DNA to prevent infection by certain viruses (bacteriophages). The enzymes were discovered in laboratory experiments when phages were used to infect bacteria other than their usual hosts. Restriction enzymes in the new host destroyed the phage DNA. Today, over 300

restriction endonucleases have been isolated and purified for use in DNA research.

DNA cleaved with restriction endonucleases produces **restriction fragment length polymorphisms (RFLPs)**. The size and number of pieces is determined by agarose gel electrophoresis.

Table 1. Recognition sequences of some restriction endonucleases

Enzyme	Bacterial source	Recognition sequence
<i>Bam</i> H I	<i>Bacillus amyloliquefaciens</i> H	G↓GATCC CCTAG↑G
<i>Eco</i> R I	<i>Escherichia coli</i> RY13	G↓AATTC CTTAA↑G

## Materials

restriction buffer  
micropipettes and tips  
electrophoresis buffer  
gel tray  
agarose  
reaction tubes  
tracking dye  
ethidium bromide  
DNA samples  
restriction enzyme (*Eco*RI)  
transilluminator  
camera and film

## Procedure

*Every pair of students will perform five digests: One each from the Unknown and four species.*

### A. Digestion

1. Label 5 reaction tubes: Unknown, 1, 2, 3, 4.
2. To each tube add 5  $\mu$ l restriction buffer. Using a new pipette tip, add 4  $\mu$ l restriction enzyme to each tube.
3. Add the DNA samples to each tube as follows. Use a different pipette tip for each sample. Why? \_\_\_\_\_

Tube	DNA
U	Unknown, 4 $\mu$ l
1	Organism 1, 4 $\mu$ l
2	Organism 2, 4 $\mu$ l

3	Organism 3, 4 $\mu$ l
4	Organism 4, 4 $\mu$ l

4. Centrifuge the tubes for 1 to 2 sec to mix. *Be sure to balance the centrifuge.* Then incubate in a 37°C water bath for 45 min. During incubation, prepare your minigel as described in Part B.
5. Add 1  $\mu$ l of tracking dye to each tube. Centrifuge the tubes for 1 to 2 sec to mix. *Be sure the centrifuge is balanced.*

### B. Electrophoresis of DNA samples

1. Use the molten agarose to pour a minigel as demonstrated. Allow the gel to completely harden before removing the well-forming comb. Then remove the comb and transfer the gel to an electrophoresis chamber. Submerge the gel in electrophoresis buffer.
2. Load 15  $\mu$ l of one of your samples prepared above into a well. Load your other sample.
3. Once the wells have been filled, apply power (125v) to the chamber. During the run, you will see the tracking dye migrate. Run the gel until the dye is near the end of the gel, then turn the current off and remove the gel.
4. Using a spatula, transfer the gel to the ethidium bromide staining tray for 5 to 10 min.

#### Do not touch the ethidium bromide.

Transfer the gel to distilled water to rinse for 5 min.

5. Place the gel on the transilluminator to see and photograph the DNA fragments.

#### Do not turn on the ultraviolet lamp until the plastic lid is closed.

6. Carefully draw the location of the bands from each of the digests.

**Molecular Epidemiology:  
DNA Fingerprinting**

Name \_\_\_\_\_  
Date \_\_\_\_\_

**Purpose** \_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

**Data**

Draw your gel so you have all five digests represented. Label the contents of each lane.

**Conclusion**

What is the identity of your unknown?  
How can you tell?

**Questions**

1. Differentiate between RFLP, PCR, and gene.
2. How could you use this technique to trace the source of *E. coli* O157:H7?
3. Why would you want to trace *E. coli*?
4. Viruses are very difficult to culture in a laboratory. How could this technique be used to find the source of a rabies virus?

