TODAY’S EXPERIMENT: PCR

Fern Tsien, PhD
Genetics
LSUHSC
What is PCR?

- The **polymerase chain reaction** (PCR) is a fast technique used in many labs to "amplify" or copy small segments of DNA.
- It is one of the most important scientific advances in molecular biology.
- Its creator, Kary B. Mullis, was awarded the Nobel Prize for Chemistry in 1993.
What is PCR used for?

- Once amplified, the DNA produced by PCR (PCR product) can be used in many different laboratory procedures.
- DNA fingerprinting in forensics and paternity testing
- Detection of bacteria or viruses (HIV/AIDS)
- Diagnosis of genetic disorders
- Many types of research!
PCR: Polymerase Chain Reaction

- Used to make millions of exact copies of DNA from a biological sample
- Allows very small samples to be analyzed, such as a sample of a few skin cells
- Must be very careful about contamination in this process
Inside the PCR reaction tube:

- DNA that you want to analyze
- Primers (little pieces of DNA that define the area that you want to analyze)
- Nucleotides (A, T, C, G)
- Taq polymerase (drives this entire reaction)
- Magnesium, salts, etc. (for solution stability)
- Sterile distilled water
The DNA is amplified millions of times in a PCR machine. The PCR machine heats and cools the DNA, primers, and other reagents in the tube and allows the amplification to take place. This results in millions of copies.
Figure S-2: Gel Electrophoresis

1. Restriction enzymes cleave DNA into smaller segments of various sizes.

2. DNA segments are loaded into wells in a porous gel. The gel floats in a buffer solution within a chamber between two electrodes.

3. When an electric current is passed through the chamber, DNA fragments move toward the positively-charged cathode.

4. Smaller DNA segments move faster and farther than larger DNA segments.
Electrophoresis

- You will dilute the electrophoresis buffer (salt solution-TBE or TAE).
- Do not use the concentrated 10X solution!
- Dilute 10X TBE to 1XTBE
  - Ratio of 1:10
  - 1 ml of 10XTBE + 9 ml of ddH2O
    = 10 ml of 1XTBE
- Never run a gel in water!! It will not work and can damage the equipment!!
- Also be careful with the electric current.
Gel electrophoresis analysis

Figure 10-30 part 2 of 2  Essential Cell Biology, 2/e. (© 2004 Garland Science)
Who did it????
DNA will be used to identify the culprit

- **Crime Scene** Samples A, B, C, and D:
  - DNA from blood found in the crime scene.

- **Suspect 1 (Smith)** Sample E:
  - DNA was obtained from a blood sample from Mr. Smith

- **Suspect 2 (Plant)** Sample F:
  - DNA was obtained from a blood sample from Mr. Plant
WHO DID IT ???

Do your experiment to find out!

Refer to your protocol and lab instructors.