

Biology AP : Virtual Bacterial Identification

Purpose:

The purpose of the lab is to familiarize you with the science and techniques used to identify different types of bacteria based on their DNA sequence. Not long ago, DNA sequencing was a time-consuming, tedious process. With readily available commercial equipment and kits, this process is now routine. The PCR (Polymerase Chain Reactions) techniques used in this lab are applicable in a wide variety of settings, including scientific research and forensic labs.

Basic Steps

- Prepare a sample from a patient and isolate whole bacterial DNA.
- Make many copies of the desired piece of DNA.
- Sequence the DNA.
- Analyze the sequence and identify the bacteria.

The piece of DNA used for identifying bacteria is the region that codes for a small subunit of the ribosomal RNA (16S rRNA). We will refer to this piece as 16S rDNA. Different bacterial species have unique 16S rDNA sequences. The identification relies on matching the sequence from your sample against a database of all known 16S rDNA sequences.

Learning Objectives

- What kind of patient samples are used for the purpose of identifying possible pathogens?
- What does PCR do, how does it work, and why is it useful?
- How do you separate the desired DNA from all others?
- How does an automatic DNA sequencer work?
- Why is it possible to use a DNA sequence to identify bacteria?

Throughout each exercise (there are total 6 parts), a laboratory window and accompany text references will guide you. The text will give you information which explains what you are doing. All the interactions, however, will be done inside the graphic window to the left of the text window. The small white box below the lab graphic will give you specific instructions on what objects to click on. You do not need to do lab simulation at once, but is it best to do so. The accompanying questions can be done after or before you do the simulation.

To run this simulation you will need the latest version of **Shockwave**. It can be downloaded from the virtual laboratory site.

Virtual Lab Site

To find the virtual site follow one of the following three pathways:

1. At the Hughes Medical Center web site (<http://www.hhmi.org>) type *Virtual Bacterial Identification* in the search box. In the window select *HHMI Virtual Bacterial ID Lab*.

2. Type the following URL: http://www.hhmi.org/biointeractive/vlabs/bacterial_id/index.html
3. Or finally in Google type in *Virtual Bacterial Identification Lab*. It should be one of the first few sites to appear on the list.

Questions and Required Work

On a separate of paper answer the following questions. You will also be required to print out and attach several pages.

Introduction

1. What is the purpose of this laboratory?
2. What sequence is being decoded?
3. What is a Sevedberg unit? What does it depend upon?

In the following the first set of questions refer to the procedures of the virtual lab, while the second set refer to the background reading for this part of the lab. You can read the background information before or after completing each part of the lab. The virtual lab takes approximately 30-40 minutes to complete. Reading the background information will require additional time. Plan to spend up to 60 minutes for reading and answering all questions.

Part 1 – Sample Preparation

1. How long does it take to “digest” the DNA?
2. Where (in the tube) is the DNA after centrifugation?
3. What enzymes are removed in this part of the laboratory?
4. How are these enzymes denatured?

Part 2 – PCR Amplification

1. After the 30th cycle how many copies of DNA are made? Be exact with your answer!
2. What is a thermocycler?
3. What does it mean to “anneal” the DNA? Why does the DNA need to be “cooled” for this step?
4. What is the origin of the DNA polymerases for this technique?

Part 3 – PCR Purification

1. After the first centrifugation of the DNA what need to be done?
2. Why is it necessary to run a gel at this point in the process?
3. Nowadays it is always necessary to run a gel? Why or why not?

Part 4 – Prepare for Sequencing

1. What are the colors of the fluorescence-tagged terminators?
2. What is the purpose of the dideoxynucleotides?