

Virtual PCR Bacterial Identification w/BLAST

Purpose:

The purpose of the lab is to familiarize you with the science of PCR (Polymerase Chain Reactions) amplification and identification of different bacteria based on their DNA sequence. The PCR techniques used in this lab are applicable to a wide variety of settings, including scientific research and forensic labs.

Background:

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

Virtual Site Lab:

To find the virtual site follow one of these pathways.

1. Go to Howard 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 use my weblink on fmfranco.com

On a separate sheet of paper answer the following questions. Plan on 30-60 minutes to complete the lab and answer all questions. Be prepared to print results.

Introduction:

1. What is the purpose of this laboratory?
2. What sequence is being decoded?
3. What is a Sevedberg Unit?
4. What does a Sevedberg Unit depend on?

Part 1- Sample Preparation

1. How long does it take to “digest the DNA?”
2. Where (in the tube) is the DNA after the 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?

4. Why does the DNA need to be “cooled” for this step?
5. What is the origin of the DNA polymerase for this technique?

Part 3- PCR Purification

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

Part 4- Preparing for Sequencing

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

Part 5- DNA Sequencing

1. What is the charge of a DNA molecule?
2. What nucleotide does each fluorescent tag represent? (match color to letter)

Part 6- Sequencing Analysis

1. Print your data sequence page.
2. Print your result page (after identifying your bacteria)
3. Attach printed pages to you answer page.