BLAST the invader: Solving respiratory illness mysteries

How do medical scientists identify the causal agent of respiratory disease? What tools allow medical researchers to decode a nucleotide sequence and match it to a specific pathogen?

In this activity, you will take on the role of a bioinformatician at a diagnostic lab. Your task is to analyze a nucleotide sequence obtained from a patient with a respiratory infection and determine which organism is causing the symptoms. By comparing your sequence to publicly available databases, you will identify the pathogen and gain insight into its classification, biology, and what general treatment options may be effective.

Case study: A mystery illness in an Ohio resident

A 19-year-old patient has been admitted to OSU-Wexner Medical Center in respiratory distress, including severe shortness of breath, fever, fatigue, and a persistent cough. The radiologist confirmed inflammation in the lungs by chest X-rays, and the hematologist reported elevated white blood cell counts, suggesting an infection. However, the exact cause remains unclear.

The patient is provided supplemental oxygen and other supportive care in the Intensive Care Unit. A sample of mucus from the patient's respiratory tract was rushed to the hospital's molecular diagnostics lab for analysis. Using advanced sequencing technologies that covered all possible barcodes for pathogens, a nucleotide sequence from the causal agent was isolated from the sample. Your job, as part of the diagnostic research team, is to use bioinformatics to identify the organism responsible for this respiratory infection.

You will use the National Center for Biotechnology Information (NCBI) website and the program BLAST (basic local alignment tool) to determine which pathogen is a close match to yours. The information you uncover will help the medical team provide accurate treatment for the patient and possibly alert public health officials if the pathogen poses a broader risk to the community.

Materials

- A computer with internet access
- Nucleotide sequence provided by your teacher

Procedure

1. Visit the NCBI website at ***ncbi.nlm.nih.gov***.
2. On the homepage, look for the "BLAST" option in the right-hand menu and click it.
3. Select "Nucleotide BLAST" from the list of available tools.
4. Copy and paste the sequence you've been assigned into the large text box labeled "Enter Query Sequence." *(Be sure to include the entire sequence, including the* **>xxx***.)*

5. Under “Database,” ensure the option is set to Standard databases (nr etc.)
6. Scroll down and click the blue “BLAST” button.
7. Wait for the BLAST program to complete the search.
8. At the bottom of the results page, there will be a section called “Descriptions” with a table listed as “Sequences producing significant alignments.” These are the matches (called “hits”) of your sequence to known nucleotide sequences in the database.
9. Click on the description to see the alignment between your sequence and the database sequence.
10. Record the organism’s name and other relevant details provided by the alignment shown.
11. Click on the unique “Sequence ID” at the top of the alignment to learn more about the database sequence.

Reflection

1. Is the nucleotide sequence provided by the diagnostic lab DNA or RNA? How can you tell?

2. Which pathogen does your sequence belong to?

3. What is the pathogen’s classification (bacterium, fungus, or virus)?

4. What is the percent identity (per. ident) between your (query) sequence and the top (subject) sequence match? (Hint: It is shown within a column in the Description table.)

5. What is the Sequence ID for the top match? (Hint: See Accession number in the Description table or Alignment page.)

6. Research information about your assigned pathogen. Prepare a presentation to address the following questions:
   - What treatments are typically recommended for this pathogen?
   - Could this pathogen pose a broader public health risk? Why or why not?
   - Are there preventive measures to avoid infections by this pathogen?