**Investigating Sequence Similarity**

**Exercise 4.2: Identification of Pathogenic Species in Horse Corneal Ulcers**

**Instructor Preface**

This document includes:

1. A student handout for Exercise 4.3
2. A postlab worksheet containing a compilation of the questions integrated throughout the student handout for Exercise 4.3 that students can complete and turn in for a post-lab assignment
3. An appendix with the FASTA files required for this project (should be provided to the students in 2 separate FASTA files)

Instructors should distribute the student handout and the question compilation worksheet to students, and make two separate FASTA file with the sequences to distribute as separate documents.

**Investigating Sequence Similarity**

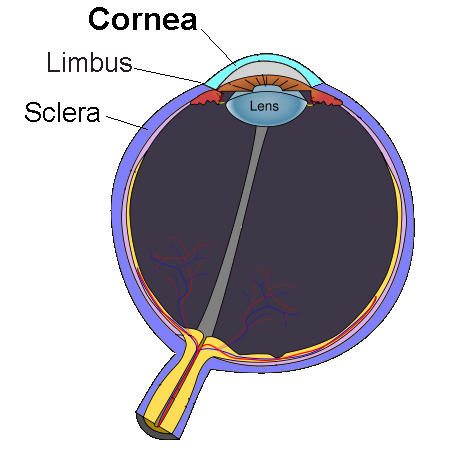
EXERCISE 4.3

**Inquiry-Based Investigation – Identification of Pathogenic Species in Horse Corneal Ulcers**

**Objectives**

After completing this exercise, you should be able to:

1. Apply bioinformatics tools to address a real-life biological question

As horses interact with their environment their eyes are susceptible to getting scratched from tree limbs, barn doors, debris kicked up on the racetrack, etc. (Wada et al., 2010). These seemingly innocuous eye injuries open the door for opportunistic pathogens such as bacteria and fungi and can result in a condition known as *ulcerative keratitis*. Ulcerative keratitis is an inflammation of the cornea and can occur in equines (horses) or humans. The *cornea*, the eye’s clear, outermost layer (**Figure 1**) plays a role in focusing vision, is incredibly thin (~1 mm) and is particularly prone to damage in horses. Corneal disease in horses is very common and can be sight threatening, thus fast diagnosis and appropriate treatment are key. Veterinarians can use fluorescein, a bright green dye to reveal corneal puncture wounds and ulcers. This damage to the cornea can heal quickly on its own, become an infected ulcer that can be treated at home, or progress to a melting ulcer that often requires hospitalization and surgery. For clinicians, picking the appropriate drug treatment for these cases is tough. Ulcerative keratitis can be caused by bacterial or fungal or mixed infections, and numerous antibiotics and antifungals exist to combat the disease. So how do you choose the appropriate treatment?

**Figure 1**. Schematic diagram of an eye. Credit: *Mikael Haggstrom, CC Public Domain, via Wikimedia Commons.* [*https://commons.wikimedia.org/wiki/File:Cornea.png*](https://commons.wikimedia.org/wiki/File:Cornea.png)

Identification of the pathogen is a key first step in determining the therapeutic course for the animal. Revealing the microorganism responsible for the infection can be challenging and traditional methods rely on fluorescein/rose Bengal cornea staining, cultures grown from corneal samples, and cytology (scraping the ulcer and examination of cells) (Zeiss et al., 2013). Numerous antibiotics and antifungals exist to treat these infections and certain drugs may be more effective at combating infections linked to a particular organism (Lalitha et al., 2007).

You are working with a collaborative team of equine clinicians and fungal researchers to pilot the use of next generation DNA sequencing (NGS) technology to identify the microorganisms responsible for ulcerative keratitis in equine cases. You’ve been given the DNA sequences below from a series of corneal ulcer cases. The source organism is known for all of the sequences except your most recent case (labeled “clinical\_sample”).

**Your task is to**

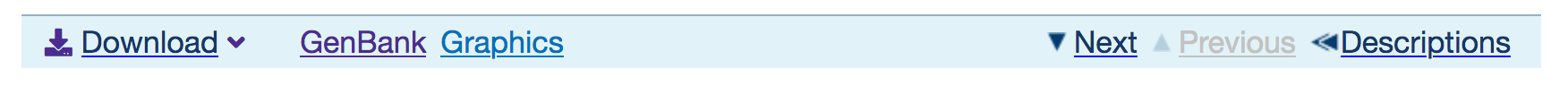
1. *determine if your clinical sample is fungal or bacterial* **(using Genbank BLAST)**
2. **Based on this initial search, create a phylogenetic tree using *either* the fungal sequence dataset or the bacterial dataset, to try to guess the species of your clinical sample**
3. *Propose a drug treatment option based on the identification of the pathogenic organism (should you use antibiotics or antifungal drugs?)*

**Corneal Ulcer Case DNA Sequence:**

>Clinical\_sample

GGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTACCGAGTGAGGGCCCTCTGGGTCCAACCTCCCACCCGTGTCTATCGTACCTTGTTGCTTCGGCGGGCCCGCCGTTTCGACGGCCGCCGGGGAGGCCTTGCGCCCCCGGGCCCGCGCCCGCCGAAGACCCCAACATGAACGCTGTTCTGAAAGTATGCAGTCTGAGTTGATTATCGTAATCAGTTAAAACTTTCAACAACGGATCTCTTGGTTCCGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAGTCTTTGAACGCACATTGCGCCCCCTGGTATTCCGGGGGGCATGCCTGTCCGAGCGTCATTGCTGCCCTCAAGCACGGCTTGTGTGTTGGGCCCCCGTCCCCCTCTCCCGGGGGACGGGCCCGAAAGGCAGCGGCGGCACCGCGTCCGGTCCTCGAGCGTATGCTCTAATTGTCACCTGCTCTGTAGGCCCGGCCGGCGCCAGCCGACACCCAACTTTATTTTTCTAAGGTTGACCTCGGATCAGGTAGGGATACCCGCTGAACTTAAGCATATCAATAAGCGGAGGA

1. Start by using **Genbank BLAST** (you should be able to find this website from previous lab exercises) and entering in this sequence data above. Once you get your BLAST results, start by clicking on the top record, then find the Genbank record by clicking on the GenBank link (see below)

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In the Genbank record, scroll down to Organism. Examine the taxonomy of the organism to see if it is **fungal or bacterial** in nature.

Answer Postlab Question 1

1. **Next,** based on whether the sample is bacterial or fungal, cut and paste your clinical sample into one of the .fas sequence datasets. Using MEGA, align the sequences in your dataset, and create a phylogeny to reveal sequence similarity and infer phylogeny.
2. Remember, after doing the alignment, export as a .MEG file to do the tree
3. I suggest doing a standard **maximum likelihood tree** with ~500 bootstrap replicates
4. Make sure the tree has **branch lengths** (all of the tips won’t line up perfectly), **Image>Save as pdf,** and save a copy of the tree to use in your postlab.

**Answer Postlab Questions 2 & 3**

**References**

Lalitha, P., Shapiro, B.L., Srinivasan, M., Prajna, N.V., Acharya, N.R., Fothergill, A.W., Ruiz, J., Chidambaram, J.D., Maxey, K.J., Hong, K.C., McLeod, S.D., Lietman, T.M. (2007). Antimicrobial Susceptibility of Fusarium, Aspergillus, and Other Filamentous Fungi Isolated From Keratitis. *Arch Ophthalmol*;125(6):789-793. doi:10.1001/archopht.125.6.789

Wada, S., Hobo, S., & Niwa, H. (2010). Ulcerative keratitis in thoroughbred racehorses in Japan from 1997 to 2008. *Veterinary Ophthalmology*, 13, 2, 99–105. doi: 10.1111/j.1463-5224.2010.00767.x.

Zeiss, C., Neaderland, M., Yang, F.C., Terwilliger, G., Compton, S., (2013) Fungal polymerase chain reaction testing in equine ulcerative keratitis. *Veterinary Ophthalmology*, 16,5, 341-351. doi: 10.1111/vop.12004.

**Investigating Sequence Similarity**

**Postlab**

**Exercise 4.3 – Inquiry-Based Investigation – Identification of Pathogenic Species in Horse Corneal Ulcers**

1. Postlab question 1: According to your BLAST results, do you suspect the clinical sample is a bacterial pathogen or a fungal pathogen? Cite specific evidence (e.g. taxonomic information from the Organism part of the record) in your answer.
2. Postlab question 2: Paste a copy of your phylogenetic tree, with branch lengths. Include a short informative caption for your figure.
3. Postlab question 3: Based on the phylogenetic groupings and the branch lengths, what can you infer from the tree about the identity of your clinical sample? Cite evidence from your tree in your answer

**Appendix 1: FASTA-formatted sequences (bacterial dataset)**

>B\_Streptococcus\_pyogenes

AGAGTTTGATCCTGGCTCAGGACGAACGCTGGCGGCGTGCCTAATACATGCAAGTAGACGAACGGGTGAGTAACGCGTAGGTAACCTACCTCATAGCGGGGGATAACTATTGGAAACGATAGCTAATACCGCATAAGAGAGACTAACGCATGTTAGTAATTTAAAAGGGGCAATTGCTCCACTATGAGATGGACCTGCGTTGTATTAGCTAGTTGGTGAGGTAAAGGCTCACCAAGGCGACGATACATAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCGGCAATGGGGGCAACCCTGACCGAGCAACGCCGCGTGAGTGAAGAAGGTTTTCGGATCGTAAAGCTCTGTTGTTAGAGAAGAATGATGGTGGGAGTGGAAAATCCACCAAGTGACGGTAACTAACCAGAAAGGGACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTCCCGAGCGTTGTCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTTTTTAAGTCTGAAGTTAAAGGCATTGGCTCAACCAATGTACGCTTTGGAAACTGGAGAACTTGAGTGCAGAAGGGGAGAGTGGAATTCCATGTGTAGCGGTGAAATGCGTAGATATATGGAGGAACACCGGTGGCGAAAGCGGCTCTCTGGTCTGTAACTGACGCTGAGGCTCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAGGTGTTAGGCCCTTTCCGGGGCTTAGTGCCGGAGCTAACGCATTAAGCACTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCCCGATGCCCGCTCTAGAGATAGAGTTTTACTTCGGTACATCGGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCCTATTGTTAGTTGCCATCATTAAGTTGGGCACTCTAGCGAGACTGCCGGTAATAAACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGGTTGGTACAACGAGTCGCAAGCCGGTGACGGCAAGCTAATCTCTTAAAGCCAATCTCAGTTCGGATTGTAGGCTGCAA CTCGCCTACATGAAGTCGGAATCGCTAGTAATCGCGGATCAGCACGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCACGAGAGTTTGTAACACCCG

>P\_aeruginosa

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>Klebsiella\_pneumoniae

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>Moraxella\_lacunata

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**Appendix 2: FASTA-formatted sequences (fungal dataset)**

>ITS1\_Aspergillus\_fumigatus

GGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTACCGAGTGAGGGCCCTCTGGGTCCAACCTCCCACCCGTGTCTATCGTACCTTGTTGCTTCGGCGGGCCCGCCGTTTCGACGGCCGCCGGGGAGGCCTTGCGCCCCCGGGCCCGCGCCCGCCGAAGACCCCAACATGAACGCTGTTCTGAAAGTATGCAGTCTGAGTTGATTATCGTAATCAGTTAAAACTTTCAACAACGGATCTCTTGGTTCCGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAGTCTTTGAACGCACATTGCGCCCCCTGGTATTCCGGGGGGCATGCCTGTCCGAGCGTCATTGCTGCCCTCAAGCACGGCTTGTGTGTTGGGCCCCCGTCCCCCTCTCCCGGGGGACGGGCCCGAAAGGCAGCGGCGGCACCGCGTCCGGTCCTCGAGCGTATGGGGCTTTGTCACCTGCTCTGTAGGCCCGGCCGGCGCCAGCCGACACCCAACTTTATTTTTCTAAGGTTGACCTCGGATCAGGTAGGGATACCCGCTGAACTTAAGCATATCAATAAGCGGAGGA

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>ITS6\_Cystrodendron

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