**Investigating Sequence Similarity**

**Exercise 4.2: Tracking a virus**

**Instructor Preface**

This document includes:

1. A student handout for Exercise 4.2
2. A postlab worksheet containing a compilation of the questions integrated throughout the student handout for Exercise 4.2 that students can complete and turn in for a post-lab assignment
3. An appendix with the FASTA files required for this project

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

**Investigating Sequence Similarity**

EXERCISE 4.2

**Inquiry-Based Investigation – Tracking a Virus**

**Objectives**

After completing this exercise, you should be able to:

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

Zika virus was first characterized in 1947 and spread through both Africa and Asia for decades before increasing dramatically in its virulence in South America in 2015. Investigate whether the 2015-2016 Brazilian Zika strain evolved from:

1. The Asian lineage, or
2. The African lineage..

… by aligning the full length viral genome sequences isolated from clinical samples, mosquitos, and apes. Your investigation will parallel the efforts of virologists seeking to understand the history of Zika as well as what molecular changes in the Zika genome have led to increased virulence (Wang et al., 2016).

To investigate this question, use your knowledge of bioinformatics tools and apply them to retrieving FASTA sequences, performing a multiple sequence alignment, and generating a phylogeny.

Answer Postlab question 1

1. Start by retrieving the FASTA genomic sequences using the **NCBI Genbank** accession numbers found in **Table 1** and paste them into the instructor provided **.fas** file. You will notice that a subpopulation of the sequences have already been imported into the .fas file to speed up your analysis.
	1. Use the Genbank accession numbers to look up the sequence
	2. To easily access the FASTA-formatted sequence, click on the “FASTA” link on the sequence record (see below). Then you can cut and paste the new sequences into your .fas file (we’ve done the first few for you).
		1. Remember to re-name your sequences something simple- we’ve included the Genbank #, the year, and the location at minimum.
		2. Remember to do this in a proper text editing program, i.e. TextMate (Mac) or NotePad (PC), to maintain it in .fas format.



1. Next, using MEGA, align the genomic sequences for the different isolated strains of Zika virus and create a phylogeny to reveal sequence similarity and infer phylogeny.
	1. Due to the relatively large size of the sequences (10,000+ bp), it may take a few minutes for MEGA to do the alignment and to make the phylogeny
	2. I suggest doing a standard **maximum likelihood tree** with ~100 bootstrap replicates (this may take a while)
	3. 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 question 2***

**Table 1.** Zika viral strain genomic sequence information associated with historical host isolation descriptive data. Sequences in **bold** are already included in your .fas file; you will have to upload the remaining from Genbank

| **Year Isolated** | **Location** | **Host Isolated From** | **NCBI GenBank Accession #** | **Strain Name** |
| --- | --- | --- | --- | --- |
| **1947** | **Uganda**  | **Monkey****(*Macaca mulatta*)** | **LC002520** | **MR766-NIID (Reference Strain)** |
| **1966** | **Malaysia** | **Mosquito** **(*Aedes aegypti*)** | **HQ234499** | **P6-740** |
| **1976** | **Central African Republic** | **Mosquito** **(*Aedes africanus*)** | **KF268948** | **ARB13565** |
| **2001** | **Senegal** | **Mosquito****(*Aedes dalzieli*)** | **KF383118** | **ArD157995** |
| 2007 | Micronesia | Human | EU545988 | FSM |
| 2015 | Brazil | Human | KU321639 | ZikaSPH2015 |
| 2016 | Brazil | Human | KU926309 | Rio-U1 |

***Answer Postlab question 3***

**References**

Wang, L., Valderramos, S. G., Wu, A., Ouyang, S., Li, C., Brasil, P., ... & Aliyari, R. (2016). From mosquitos to humans: genetic evolution of Zika virus. *Cell Host & Microbe*, 19(5), 561-565. doi: 10.1016/j.chom.2016.04.006.

**Investigating Sequence Similarity**

**Postlab**

**Exercise 4.2 – Inquiry-Based Investigation – Tracking a Virus**

1. Postlab question 1: The intro proposed 2 hypotheses (4 pts):

H1: The 2015-2016 Brazilian Zika strain evolved from the Asian lineage of Zika

H2: The 2015-2016 Brazilian Zika strain evolved from the African lineage of Zika

What would you expect a phylogeny of the Zika genomes to look like under hypothesis 1 vs hypothesis 2? Explain in words.

Postlab question 2: Upload a pdf of your tree and refer to it in your answer, **annotating on the tree** the clade with the 2015-16 Brazilian Zika genomes

P*ostlab question 3. Do the data support one of the proposed hypotheses? Explain, clearly referring to the tree in Postlab Q2 (3 pts)*

**Appendix 1. FASTA-formatted sequences for this exercise**

>LC002520\_1947\_Uganda\_Monkey\_Zika\_MR766-NIID

AGTTGTTGATCTGTGTGAGTCAGACTGCGACAGTTCGAGTCTGAAGCGAGAGCTAACAACAGTATCAACA

GGTTTAATTTGGATTTGGAAACGAGAGTTTCTGGTCATGAAAAACCCAAAGAAGAAATCCGGAGGATTCC

GGATTGTCAATATGCTAAAACGCGGAGTAGCCCGTGTAAACCCCTTGGGAGGTTTGAAGAGGTTGCCAGC

CGGACTTCTGCTGGGTCATGGACCCATCAGAATGGTTTTGGCGATACTAGCCTTTTTGAGATTTACAGCA

ATCAAGCCATCACTGGGCCTTATCAACAGATGGGGTTCCGTGGGGAAAAAAGAGGCTATGGAAATAATAA

AGAAGTTCAAGAAAGATCTTGCTGCCATGTTGAGAATAATCAATGCTAGGAAAGAGAGGAAGAGACGTGG

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GAGTGAACAAGTGCCACGTACAGATCATGGACCTCGGGCACATGTGTGACGCCACCATGAGTTATGAGTG

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