GDSCN Book: SARS Phylogeny on AnVIL

October 11, 2022

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# Overview

This book provides resources for instructors to engage students in a cloud-based RStudio activity on AnVIL, focused on the evolutionary relationships among the SARS-CoV-2 variants.

There is a growing need for undergraduate students to learn cutting-edge concepts in genomics data science, including performing analysis on the cloud instead of a personal computer. This lesson aims to introduce basic tree building and interpretation using publicly available genetic samples of SARS-CoV-2. Students will be introduced to the sequencing revolution, variants, the basics of tree building and reading phylogenies, and essentials of cloud computing prior to the lab activity. During the lesson, students will work hands-on with RStudio on the [AnVIL](https://anvilproject.org/) cloud computing resource to check data, build trees, and visualize their results.

## Skills Level

*Genetics*  
**Beginner**: minimal genetics knowledge needed

*Programming skills*  
**Beginner**: minimal programming experience needed

## Learning Objectives

Learning objectives for this activity come from the [Genetics Core Competencies](https://genetics-gsa.org/education/genetics-learning-framework/):

* Generate and interpret trees displaying experimental results
* Use bioinformatics to assess genetics data
* Tap into the interdisciplinary nature of science

## AnVIL Collection

Please check out our full collection of AnVIL resources below!

Book Name

Description

Topics

[AnVIL Phylogenetic-Techniques](https://jhudatascience.org/AnVIL_Phylogenetic-Techniques/) ([github](https://github.com/jhudsl/AnVIL_Phylogenetic-Techniques))

<https://jhudatascience.org/AnVIL_Phylogenetic-Techniques/>

anvil

[AnVIL: Getting Started](https://jhudatascience.org/AnVIL_Book_Getting_Started) ([github](https://github.com/jhudsl/AnVIL_Book_Getting_Started))

A guide for getting started using AnVIL

anvil, cloud-computing

[AnVIL: Instructor Guide](https://jhudatascience.org/AnVIL_Book_Instructor_Guide) ([github](https://github.com/jhudsl/AnVIL_Book_Instructor_Guide))

A guide for instructors using AnVIL for workshops, lessons, or courses.

anvil, education

[GDSCN: SARS Galaxy on AnVIL](https://jhudatascience.org/GDSCN_Book_SARS_Galaxy_on_AnVIL/) ([github](https://github.com/jhudsl/GDSCN_Book_SARS_Galaxy_on_AnVIL))

Lab module and lectures for variant detection in SARS-CoV-2 using Galaxy

anvil, genomics, module

[GDSCN: Statistics for Genomics Differential Expression](https://jhudatascience.org/GDSCN_Book_Statistics_for_Genomics_Differential_Expression/) ([github](https://github.com/jhudsl/GDSCN_Book_Statistics_for_Genomics_Differential_Expression))

A set of lab modules for an introduction to differential gene expression

anvil, cloud-computing, gene-expression

[GDSCN: Statistics for Genomics PCA](https://jhudatascience.org/GDSCN_Book_Statistics_for_Genomics_PCA/) ([github](https://github.com/jhudsl/GDSCN_Book_Statistics_for_Genomics_PCA))

A set of lab modules for PCA analysis

anvil

[GDSCN: Statistics for Genomics RNA-seq](https://jhudatascience.org/GDSCN_Book_Statistics_for_Genomics_RNA-seq/) ([github](https://github.com/jhudsl/GDSCN_Book_Statistics_for_Genomics_RNA-seq))

A set of lab modules for RNA-seq analysis

anvil

[GDSCN: Statistics for Genomics scRNA-seq](http://jhudatascience.org/GDSCN_Book_Statistics_for_Genomics_SCRNA-seq/) ([github](https://github.com/jhudsl/GDSCN_Book_Statistics_for_Genomics_scRNA-seq))

A set of lab modules for single cell RNA-seq analysis

anvil

# Instructor Guide

## Suggested Activity Context

**Course Audience**

* Undergraduate biology majors
* Graduate students with less exposure to bioinformatics

**Course Prerequisites**

* Layman understanding of genetics (understanding of DNA, genes, trait inheritance)
* Some previous exposure to the central dogma of molecular biology

**Class Type**

* Lab
* Computer-based

**Class Size**

* 1-50

**Lesson Duration**

Coming soon!

**Assessment Type**

* Short answer questions at each lab stage

## Lesson Plan

Coming soon!

## Notes for Instructors

Coming soon!

## Getting Credit for Professional Development

We are happy to provide a letter to your supervisor, department head, or dean to indicate you’ve worked through this content and intend to use it in your class. .

# 1 What Is a Variant?

This lecture module introduces genetic variants. It provides several examples of genetic variants, background on the structure of DNA, and a review of the “Central Dogma” of molecular biology - the process of turning DNA into RNA into protein.

**Learning Objectives**

1. Answer “what is a genetic variant?”
2. Learn about the molecular structure of a variant

You can view and download the Google Slides [here](https://docs.google.com/presentation/d/1097pZ-m7u22TBs6vqgbqPT1TH4FwdCY4P89WLTp2wT4/edit?usp=sharing).

# 2 The Sequencing Revolution

This lecture module introduces the history of the sequencing revolution. It highlights the enormous proliferation of genomic data that has accompanied the rapidly growing technology. It also suggests opportunities for careers in genomics, as well as an in-depth look at how some sequencing technologies actually work.

**Learning Objectives**

1. Learn the history the sequencing revolution
2. Introduce the sequencing workforce
3. Explore the evolution of sequencing technology

You can view and download the Google Slides [here](https://docs.google.com/presentation/d/1oWzck0r3djLUkS8t29v0D32-fnZDa7Qcb6GrO_VfFmk/edit?usp=sharing).

# 3 Reading Phylogenies

This lecture module introduces how to interpret phylogenetic trees and basic phylogenetic concepts.

**Learning Objectives**

1. Learn what a phylogeny is.
2. Understand how to identify most recent common ancestor between taxa.
3. Learn how to interpret phylogenetic topologies and relationships.

You can view and download the Google Slides [here](https://docs.google.com/presentation/d/1V7ZX2pK6uySy2cgQXj4GRzjI5iyKbKiK8oa0967VE9s/edit#slide=id.p).

# 4 Student Activity Guide

This chapter contains the student instructions for the SARS-CoV-2 Phylogeny with RStudio activity.

## 4.1 Introduction

Since the beginning of the Covid-19 pandemic in December 2019, the world has experienced waves of increased cases caused by new variants. This activity walks you through a simple phylogeny to explore how the new variants are related. If you are interested in learning more about this topic, we recommend you check out the SARS-CoV-2 resources on [Nextstrain.org](https://nextstrain.org/sars-cov-2/).

### 4.1.1 Before You Start

If you do not already have a Google account that you would like to use for accessing Terra, [create one now](https://accounts.google.com/SignUp).

If you would like to create a Google account that is associated with your non-Gmail, institutional email address, follow [these instructions](https://support.terra.bio/hc/en-us/articles/360029186611).

### 4.1.2 Objectives

This activity will teach you how to use the AnVIL platform to:

1. Get started working on AnVIL
2. Launch RStudio
3. Import data into RStudio
4. Examine fasta and phyDat files
5. Build a neighbor-joining phylogeny
6. Interpret the topology and branch lengths of a phylogeny

## 4.2 Getting Started

In the next few steps, you will walk through how to get set up to use RStudio on the AnVIL platform. AnVIL is centered around different “Workspaces”. Each Workspace functions almost like a mini code laboratory - it is a place where data can be examined, stored, and analyzed. The first thing we want to do is to copy or “clone” a Workspace to create a space for you to experiment.

Use a web browser to go to the AnVIL website. In the browser type:

anvil.terra.bio

**Tip** At this point, it might make things easier to open up a new window in your browser and split your screen. That way, you can follow along with this guide on one side and execute the steps on the other.

A workspace for this activity on AnVIL coming soon!

### 4.2.1 Set Up

Coming soon!

## 4.3 Exercise One: Loading libraries in RStudio

Before we can start our analysis of how the SARS-CoV-2 variants are related to each other, we need to prepare the RStudio workspace and load the data.

R is an open-source statistical programming language and anyone can contribute to it. People have written programs in R to do a ton of different things, and they can make those programs (known as packages, or libraries) available to everyone. Generally, when someone has created a package they want to share, they will submit it to a repository, where anyone using R can download it.

For this lesson, we are using the [CRAN](https://cran.r-project.org/) (Comprehensive R Archive Network) repository. There are a series of servers around the world that store the up-to-date packages. When you open R, you can access those servers and download any package you want. If we are downloading a package that has been stored on CRAN, we use the command install.packages.

We need to install two packages: [ape](https://academic.oup.com/bioinformatics/article/20/2/289/204981) and [phangorn](https://academic.oup.com/bioinformatics/article/27/4/592/198887). Both of these packages were written specifically for phylogenetic analysis in R.

To install the packages, we type the following code into the RStudio console:

install.packages('ape')  
  
install.packages('phangorn')

Once you’ve downloaded a package, it will be saved on your computer (or, in the case of AnVIL, on your persistent disk space) so that you don’t have to download it again. Anytime you want to use the set of commands that are stored in a particular package, you’ll tell R to open the package with the library command.

Let’s open both packages now:

library(ape)  
library(phangorn)

You can verify that both packages have been loaded by looking at the Packages tab in the lower left-hand window of the RStudio interface. Packages that have been loaded are checked. You can search specifically for each package, or scroll down the entire list.

## 4.4 Exercise Two: Examining fasta files in RStudio

Now we need to retrieve the data. We’ll start by loading a type of data file called a fasta file. The fasta format is a common way to store sequences (either DNA or protein). Each sample in a fasta file has two sections. The sample ID and other descriptive information is on the first line (the description line). This line begins with either a > or a ;. The sample sequence is on the line immediately after the description. The sequence is written in standard IUCAC codes for either nucleic acids (for DNA sequence) or amino acids (for protein sequence). The sequence can also include unknown bases or gaps.

The fasta file we’re loading first contains the aligned sequences for the spike protein of 5 SARS-CoV-2 samples. This is what the top of the file looks like in a text editor:

We can load this file into RStudio using the read.FASTA command and save it as the object “spike.fasta”.

spike.fasta <- read.FASTA("sars\_spike\_protein.fasta")

After we’ve created an object in RStudio, we can get information about the object by typing the object’s name.

spike.fasta

## 5 DNA sequences in binary format stored in a list.  
##   
## All sequences of same length: 3827   
##   
## Labels:  
## alpha  
## beta  
## delta  
## gamma  
## Wuhan\_reference  
##   
## Base composition:  
## a c g t   
## 0.294 0.188 0.184 0.333   
## (Total: 19.14 kb)

Here we can see a summary of what this object contains, as well as how long the sequences are and the sequence names.

Notice that RStudio has saved the information in the fasta file as binary data. This means the sequence information has been converted from “ATCG” into something easier for RStudio to work with, but harder for humans to interpret.

The phangorn package uses a special data format called phyDat, which is derived from the fasta format. A phyDat object provides some additional information about the samples we upload.

spike.phydat <- read.phyDat("sars\_spike\_protein.fasta", format = "fasta")  
  
spike.phydat

## 5 sequences with 3827 character and 46 different site patterns.  
## The states are a c g t

Different site patterns refers to sites that differ between sequences. In this small dataset, 46 of the 3827 possible bases (characters) show differences among these SARS-CoV-2 samples.

We’ve been working with a dataset that contains the original SARS-CoV-2 sequence (the Wuhan reference sample), as well as samples of the alpha, beta, delta, and gamma variants. Another variant, the omicron variant, was first identified in late 2021 and quickly became a variant of concern. Let’s look at a dataset that contains additional omicron samples.

spike.omicron <- read.phyDat("sars\_spike\_protein\_omicron.fasta", format = "fasta")  
  
spike.omicron

## 9 sequences with 3827 character and 96 different site patterns.  
## The states are a c g t

QUESTIONS:

1. What is some information saved in a .fasta object that RStudio tells us that we don’t get from a .phyDat object?
2. How many omicron sequences are there in the second file (loaded into the object spike.omicron)?
3. Do you think there is more variability in the omicron sequences than in other variants (alpha, beta, delta, and gamma)? Why or why not?

## 4.5 Exercise Three: How are variants related to the original SARS-CoV-2 reference strain?

We’ll work with both the smaller spike protein dataset (saved as spike.phydat) and the spike protein dataset with omicron samples (saved as spike.omicron) in this activity.

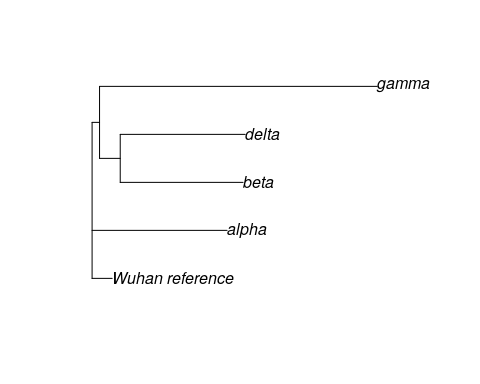
### 4.5.1 Building a neighbor-joining tree

We can use phyDat objects to build a type of phylogenetic tree called a neighbor-joining tree. We can do this in two steps in RStudio using the phangorn package.

dist.small <- dist.ml(spike.phydat)  
nj.small <- nj(dist.small)

The first command (dist.ml) converts the genetic data into a distance matrix, while the second command (nj) uses the matrix to group taxa based on the genetic distance between them. We plot the object created by the nj command to visualize our phylogenetic tree.

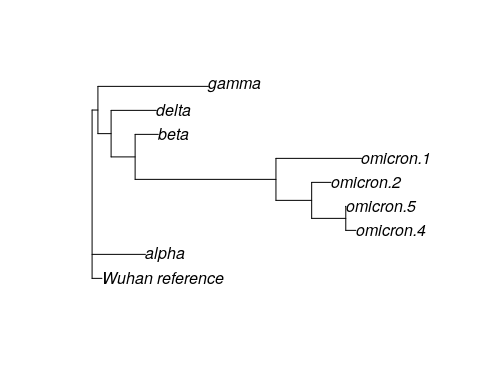
plot(nj.small)



When reading phylogenetic trees, it’s important to remember that relatedness is determined by the number of nodes between two taxa. The delta variant is most closely related to the beta variant because they share a common node (where the branches join together). We always look at common ancestors of clades, so we essentially read ancestry on a tree from right (where the taxa are) to left. Thus, the delta variant is more closely related to the beta variant because the delta branch connects to a node shared by the beta branch before it connects to a node shared with the gamma branch or the Wuhan reference/alpha variant branch.

Now let’s look at where the omicron sequences are in the SARS-CoV-2 tree.

dist.omicron <- dist.ml(spike.omicron)  
nj.omicron <- nj(dist.omicron)  
plot(nj.omicron)



QUESTIONS:

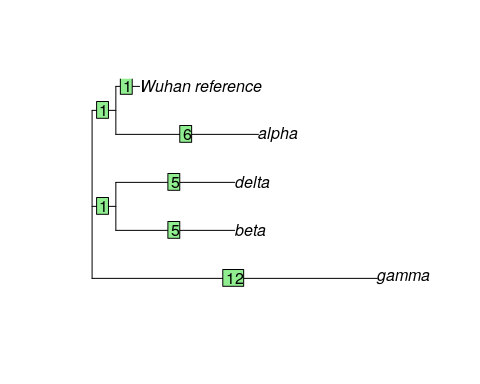
1. Which variant is most closely related to the original Wuhan reference sequence?
2. Which variant(s) is the most distantly related to the original Wuhan reference sequence?
3. Which variant shares the most recent common ancestor with the omicron variants? What does this mean for determining where the omicron variant came from?

### 4.5.2 Examining branch lengths

Now we will add branch lengths to our trees. Information about branch lengths are saved in our previous trees, but those branch lengths are equal to the number of substitutions per site, which can be difficult to interpret. Instead, we will look at trees where the branch lengths have been scaled so that each branch is equal to the total number of substitutions (changes in the DNA sequence).

First, let’s load and view a tree containing the five non-omicron sequences.

spike.tree\_small <- read.tree("sars\_tree\_small.tre")  
  
plot(spike.tree\_small)  
edgelabels(spike.tree\_small$edge.length)



The numbers in green boxes represent the number of DNA changes along a particular branch. For example, the Wuhan reference sequence is estimated to have only one DNA change from the spike protein sequence of the most recent common ancestor of all SARS-CoV-2 strains, while the alpha variant has 6 bases different from the most recent common ancestor. This also means the distance between Wuhan reference sequence and the alpha sample is 7 substitutions (1 substitution for the Wuhan sample + 6 substitutions for the alpha variant sample).

**Breakout Box: Learn more about phylogenetically informative sites**

You may be wondering why the total branch length changes (31) is different from the number of site patterns listed in the phyDat summary (46). This is because only some of the 46 site patterns are what we call *phylogenetically informative*.

Let’s look at a short example:

cat: GC**A** TT**C**

dog: GC**C** TT**C**

frog: GC**C** TT**A**

snake: GC**C** TT**A**

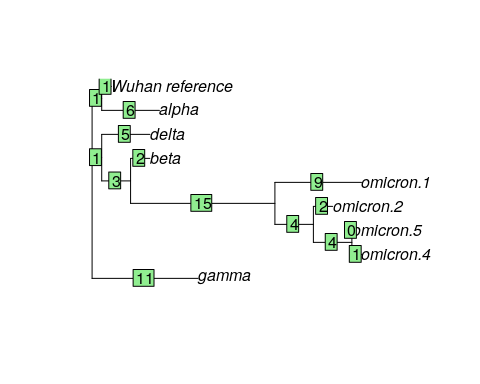
In this collection of sequences, there are 2 site patterns (both in bold), but only one is phylogenetically informative because it tells us about the relatedness between the species. While the cat sequence is different from the others at the third site, this doesn’t actually give us information about the relationships among the four species. The sixth site, however, tells us that the cat and dog share a mutation that frog and snake do not. We can interpret that to mean the cat and dog are more closely related to each other than either is to the frog or the snake. Thus, this particular site pattern gives us information we can use to build the phylogenetic tree.

QUESTIONS:

1. what is the branch length distance between the beta variant and the alpha variant?
2. What is the longest branch length in the tree? What does this mean for the number of mutations (compared to the Wuhan reference sequence) seen in that variant versus the others?

Now let’s load and view a tree containing all nine SARS-CoV-2 samples.

spike.tree\_full <- read.tree("sars\_tree\_full.tre")  
  
plot(spike.tree\_full)  
edgelabels(spike.tree\_full$edge.length)



What do these branch lengths tell us about the relationship among the SARS-CoV-2 variants?

QUESTIONS:

1. Did adding the omicron samples change the branch length distances between the original five samples? What is the branch length distance between the alpha and beta variants now?
2. What is the length of the branch connecting the omicron group to the rest of the tree
3. The covid vaccines were originally designed based on the Wuhan reference sequence of the spike protein. The immune system learns to recognize the spike protein from the vaccine and can identify and destroy any invading Covid-19 viruses. Using what you have learned about the phylogenetic tree of SARS-CoV-2 variants, can you explain why these initial vaccines were less effective at protecting against the omicron variants than they were against the delta variant?

## 4.6 Exercise Four: Do other protein-coding regions show us the same phylogenetic relationships?

The SARS-CoV-2 genome contains 6 protein-coding regions. So far, we’ve been working with genetic data from the region that codes for spike protein. (The spike protein is the part of the virus that sticks out to form the characteristic spikes on the outside of the SARS-CoV-2 virus.) However, you could also use any of the other 5 protein-coding regions for this exercise.

Let’s look at sequences from the region that codes for the membrane glycoprotein.

membrane.omicron <- read.phyDat("sars\_membrane\_protein\_omicron.fasta", format = "fasta")  
  
membrane.omicron

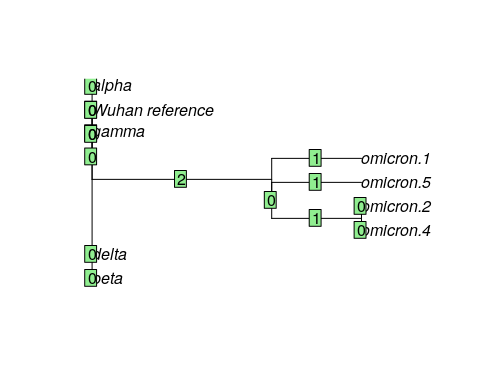
## 9 sequences with 670 character and 10 different site patterns.  
## The states are a c g t

QUESTIONS:

1. Based on the information from the phydat files for the spike protein dataset and the membrane protein dataset, is there the same amount of variation in each protein-coding region?
2. Do you think the spike protein dataset or the membrane protein dataset contains a greater number of phylogenetically-informative sites?

Now let’s look at a neighbor-joining tree built using the membrane protein dataset.

nj.membrane <- read.tree("sars\_membrane\_tree.tre")  
  
plot(nj.membrane)  
edgelabels(nj.membrane$edge.length)



QUESTIONS:

1. Is the tree built from the membrane protein data the same as the tree built from the spike protein data?
2. Why is a greater number of phylogenetically-informative sites better for tree building?

## 4.7 Wrap-up

Once you are done with the activity, you’ll need to shut down your RStudio cloud environment. This frees up the cloud resources for others and minimizes computing cost. The following steps will delete your work, so make sure you are completely finished at this point. Otherwise, you will have to repeat your work from the previous steps.

Directions coming soon!

# Appendix

# 5 Answer Guide

This page contains the answers to the reflection questions asked in the student guide. If you discover a mistake or have a suggestion for additional or alternate reflection questions, please contact us through our [Discourse Channel](https://help.anvilproject.org/) or [submit an issue on our GitHub repository](https://github.com/fhdsl/GDSCN_SARS_Phylogeny_on_AnVIL/).

1. What is some information saved in a .fasta object that RStudio tells us that we don’t get from a .phyDat object?

*The .fasta object also tells us at least some of the names of the samples in the fasta file, as well as the average base composition of all the sequences and how big the fasta file is.*

1. How many omicron sequences are there in the second file (loaded into the object spike.omicron)?

*There are four omicron sequences in the second file.*

1. Do you think there is more variability in the omicron sequences than in other variants (alpha, beta, delta, and gamma)? Why or why not?

*While adding the omicron sequences to the fasta file increases the number of site patterns in the sequences, this doesn’t necessarily mean the omicron sequences have more variability among each other than the other sequences. The omicron sequences may just be very different from the other variants.*

1. Which variant is most closely related to the original Wuhan reference sequence?

*The alpha variant is the most closely related to the original Wuhan reference sequence.*

1. Which variant(s) is the most distantly related to the original Wuhan reference sequence?

*All the variants other than the alpha variant share the same most recent common ancestor with the original Wuhan reference sequence, thus they are all equally distant relatives of the Wuhan reference sequence.*

1. Which variant shares the most recent common ancestor with the omicron variants? What does this mean for determining where the omicron variant came from?

*The beta variant shares the most recent common ancestor with the omicron variants. This suggests that the omicron variant probably evolved from an ancestor of the beta branch.*

1. what is the branch length distance between the beta variant and the alpha variant?

*The branch length distance between the beta variant and the alpha variant is 13.*

1. What is the longest branch length in the tree? What does this mean for the number of mutations (compared to the Wuhan reference sequence) seen in that variant versus the others?

*The longest branch length is 12, leading to the gamma variant. The gamma variant has a greater number of mutations compared to the Wuhan reference sequence than the others.*

1. Did adding the omicron samples change the branch length distances between the original five samples? What is the branch length distance between the alpha and beta variants now?

*Adding the omicron samples did not really change the branch lengths between the original five sequences. The distance between the alpha and beta variants is still 13.*

1. What is the length of the branch connecting the omicron group to the rest of the tree?

*The length of the branch connecting the omicron variants to the rest of the tree is 15.*

1. The covid vaccines were originally designed based on the Wuhan reference sequence of the spike protein. The immune system learns to recognize the spike protein from the vaccine and can identify and destroy any invading Covid-19 viruses. Using what you have learned about the phylogenetic tree of SARS-CoV-2 variants, can you explain why these initial vaccines were less effective at protecting against the omicron variants than they were against the delta variant?

*The distance between any of the omicron variants and the Wuhan reference variant is much larger than the distance between the delta variant and the Wuhan reference sequence. This tells us the omicron spike protein sequence has mutated more in the omicron variants, so the spike protein probably doesn’t look as much like the spike protein the vaccines train the immune system to recognize (compared to the delta variant spike proteins).*

1. Based on the information from the phydat files for the spike protein dataset and the membrane protein dataset, is there the same amount of variation in each protein-coding region?

*No, there is much less variation in the membrane protein-coding region than in the spike protein-coding region.*

1. Do you think the spike protein dataset or the membrane protein dataset contains a greater number of phylogenetically-informative sites?

*The spike protein dataset contains a greater number of phylogenetically-informative sites.*

1. Is the tree built from the membrane protein data the same as the tree built from the spike protein data?

*The tree built from the membrance protein data looks very different from the tree built from the spike protein data. The relationships are much less resolved.*

1. Why is a greater number of phylogenetically-informative sites better for tree building?

*A greater number of phylogenetically-informative sites results in more resolution among the taxa in the tree.*

# 6 Help

We welcome any and all questions at our [Discourse Channel](https://help.anvilproject.org/).

If you have feedback on the activity (Found a typo? Have a suggestion or idea?) please [submit an issue on our GitHub repository](https://github.com/fhdsl/GDSCN_SARS_Phylogeny_on_AnVIL/).

# 7 Download

Download this entire book as a Microsoft Word and Google Doc compatible docx file

Coming soon!

# 8 Give Us Feedback

Thank you for your interest in this book! There are a few ways you can suggest improvements:

Coming soon!

# 9 References

All sequences were downloaded from NCBI’s GenBank. We used sequence data from the following accession numbers: OX315675.1 (omicron.2), OP093374.1 (omicron.4), OP093373.1 (omicron.5), OK091006.1 (delta), NC\_045512.2 (Wuhan\_reference), OX003129.1 (beta), OX315743.1 (omicron.1), OW998592.1 (alpha), MW911054.1 (gamma).

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SARS-CoV-2 image credit: Davian Ho for the [Innovative Genomics Institute](https://innovativegenomics.org/free-covid-19-illustrations/)

# About the Authors

These credits are based on our [course contributors table guidelines](https://github.com/jhudsl/OTTR_Template/wiki/How-to-give-credits).

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| **Funding** |  |
| Funder | [National Human Genome Research Institute (NHGRI)](https://www.genome.gov/) |
| Funding Staff | Fallon Bachman, Jennifer Vessio, Emily Voeglein |

## ─ Session info ───────────────────────────────────────────────────────────────  
## setting value   
## version R version 4.0.2 (2020-06-22)  
## os Ubuntu 20.04.3 LTS   
## system x86\_64, linux-gnu   
## ui X11   
## language (EN)   
## collate en\_US.UTF-8   
## ctype en\_US.UTF-8   
## tz Etc/UTC   
## date 2022-10-11   
##   
## ─ Packages ───────────────────────────────────────────────────────────────────  
## package \* version date lib source   
## assertthat 0.2.1 2019-03-21 [1] RSPM (R 4.0.3)   
## bookdown 0.24 2022-02-15 [1] Github (rstudio/bookdown@88bc4ea)   
## callr 3.4.4 2020-09-07 [1] RSPM (R 4.0.2)   
## cli 2.0.2 2020-02-28 [1] RSPM (R 4.0.0)   
## crayon 1.3.4 2017-09-16 [1] RSPM (R 4.0.0)   
## desc 1.2.0 2018-05-01 [1] RSPM (R 4.0.3)   
## devtools 2.3.2 2020-09-18 [1] RSPM (R 4.0.3)   
## digest 0.6.25 2020-02-23 [1] RSPM (R 4.0.0)   
## ellipsis 0.3.1 2020-05-15 [1] RSPM (R 4.0.3)   
## evaluate 0.14 2019-05-28 [1] RSPM (R 4.0.3)   
## fansi 0.4.1 2020-01-08 [1] RSPM (R 4.0.0)   
## fs 1.5.0 2020-07-31 [1] RSPM (R 4.0.3)   
## glue 1.6.1 2022-01-22 [1] CRAN (R 4.0.2)   
## htmltools 0.5.0 2020-06-16 [1] RSPM (R 4.0.1)   
## knitr 1.33 2022-02-15 [1] Github (yihui/knitr@a1052d1)   
## lifecycle 1.0.0 2021-02-15 [1] CRAN (R 4.0.2)   
## magrittr 2.0.2 2022-01-26 [1] CRAN (R 4.0.2)   
## memoise 1.1.0 2017-04-21 [1] RSPM (R 4.0.0)   
## pkgbuild 1.1.0 2020-07-13 [1] RSPM (R 4.0.2)   
## pkgload 1.1.0 2020-05-29 [1] RSPM (R 4.0.3)   
## prettyunits 1.1.1 2020-01-24 [1] RSPM (R 4.0.3)   
## processx 3.4.4 2020-09-03 [1] RSPM (R 4.0.2)   
## ps 1.3.4 2020-08-11 [1] RSPM (R 4.0.2)   
## purrr 0.3.4 2020-04-17 [1] RSPM (R 4.0.3)   
## R6 2.4.1 2019-11-12 [1] RSPM (R 4.0.0)   
## remotes 2.2.0 2020-07-21 [1] RSPM (R 4.0.3)   
## rlang 0.4.10 2022-02-15 [1] Github (r-lib/rlang@f0c9be5)   
## rmarkdown 2.10 2022-02-15 [1] Github (rstudio/rmarkdown@02d3c25)  
## rprojroot 2.0.2 2020-11-15 [1] CRAN (R 4.0.2)   
## sessioninfo 1.1.1 2018-11-05 [1] RSPM (R 4.0.3)   
## stringi 1.5.3 2020-09-09 [1] RSPM (R 4.0.3)   
## stringr 1.4.0 2019-02-10 [1] RSPM (R 4.0.3)   
## testthat 3.0.1 2022-02-15 [1] Github (R-lib/testthat@e99155a)   
## usethis 2.1.5.9000 2022-02-15 [1] Github (r-lib/usethis@57b109a)   
## withr 2.3.0 2020-09-22 [1] RSPM (R 4.0.2)   
## xfun 0.26 2022-02-15 [1] Github (yihui/xfun@74c2a66)   
## yaml 2.2.1 2020-02-01 [1] RSPM (R 4.0.3)   
##   
## [1] /usr/local/lib/R/site-library  
## [2] /usr/local/lib/R/library