## **Nextstrain Tutorial**

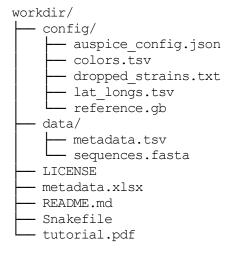
## Yale school of public health

In this tutorial we are going to generate a nextstrain.org community build. To do so, we will run a bioinformatics pipeline (augur), which will perform sequence alignment, phylogenetic reconstruction, infer ancestral states (for location and mutations), and finally combine such results to be visualized using a tool named auspice.

- (1) **Create** a folder in your directory of preference: it will be your working directory (workdir).
- (2) **Access** the repository below and **download** its content as a .zip file:

github.com/grubaughlab/nextstrain course

- (3) **Decompress** the downloaded .zip file and **copy** its content into your workdir.
- (4) Now, your workdir should look as follows:



- (5) Open the file metadata.xlsx. This spreadsheet contains three tabs, named after some of the files in workdir, they are: metadata, lat\_longs, and colors.
- (6) **Copy** and **paste** the data from each tab in the corresponding files, replacing their existing data.
- (7) The current reference.gb file is included in config/only as an example. Inspect the format of this file using a text editor, and check the fields 'CDS' and 'gene' in this annotation file. Access the link below, and download this new reference genome in .gb format (click on Send to > File > Format:Genbank > Create File).

- (8) **Open** this .gb file on a text editor, and **edit** it as follows, so that it looks like the example file inspected at step (7):
  - a. Replace 'mat\_peptide' by 'CDS'
  - b. Replace 'product' by 'gene'
  - Rename long gene names in the 'gene' fields by standard gene acronyms (see literature)
- (9) **Delete** the existing reference.gb; **move** the file created in the previous step to workdir/config, and; **rename** it as your new reference.gb file.
- (10) **Open** the file <code>auspice\_config.json</code> using a text editor and edit the lines showing the following content:
  - a. 'Add your project title here'
  - b. 'Add your name here'
  - c. 'Add your github link repository here'
- (11) **Open** the file Snakefile using a text editor, and **edit** the following lines as appropriate, keeping their suffixes ('tree' and 'meta'):
  - a. 'addHereYourProjectName\_tree.json'
  - b. 'addHereYourProjectName\_meta.json'
- (12) Having done all the edits above, access your workdir in the Terminal, and run augur using the command below:

snakemake export

- (13) **Watch** for potential error messages: they may indicate issues with file formatting in previous steps.
- (14) If no issues were flagged, now **run** auspice to visualize the results using the command below:

auspice view

(15) **Open** a web browser and **access** the results using the link generated by the command above, which may look like the url below:

http://localhost:4000