

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:

```
workdir/
├── config/
│   ├── auspice_config.json
│   ├── colors.tsv
│   ├── dropped_strains.txt
│   ├── lat_longtsv
│   └── reference.gb
├── data/
│   ├── metadata.tsv
│   └── sequences.fasta
├── LICENSE
├── README.md
├── Snakefile
├── metadata.xlsx
├── nextstrain_installation.pdf
└── nextstrain_tutorial.pdf
```

(5) **Open** the file *metadata.xlsx*. This spreadsheet contains three tabs, named after some of the files in *workdir*, they are: *metadata*, *lat_longtsv*, 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).

ncbi.nlm.nih.gov/nuccore/JX669468

(8) **Open** this *.gb* file on a text editor, and **edit** it as follows, keeping all the edited elements aligned, so that it looks like the file inspected at step (7):

- Replace** 'mat_peptide' by 'CDS'
- Replace** 'product' by 'gene'
- Rename** long gene names at 'gene' fields by standard gene acronyms (check the 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 *auspice_config.json* using a text editor and **edit** the lines showing the following content:

- 'Add your project title here'
- 'Add your name here'
- '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'):

- 'addHereYourProjectName_tree.json'
- '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>