

## Data processing procedures

1. Download input files from GISAID
  - Download the nucleotide sequences in FASTA format (e.g., FASTA.fasta).
  - Download the “Dates and Location” metadata as TSV (e.g., metadata.tsv).
2. Run Nextclade to generate a TSV file
  - Run this in Windows PowerShell:

```
mkdir out
.\nextclade-x86_64-pc-windows-gnu.exe run -D .\data FASTA.fasta --
output-tsv .\out\nextclade.tsv
```
3. Generate mutation lists for a given date range ( $\geq 50\%$  frequency)
  - Run:

```
python common_mutations_by_date.py --nextclade .\out\nextclade.tsv --
meta metadata.tsv --start YYYY-MM-DD --end YYYY-MM-DD
```

This outputs:
    - common\_nucl\_\*.csv (nucleotide substitutions)
    - common\_spikeaa\_\*.csv (Spike amino-acid substitutions)
4. Compare two mutation lists (A vs B)
  - Run:

```
python compare_mut_lists.py --A A.csv --B B.csv
```

(A.csv and B.csv should be mutation list files generated in Step 3, e.g., nucleotide lists or Spike AA lists.)
5. Run the python program available from the GitHub to generate a comparison list.
  - If you want to count substitution types (e.g., C→U, G→A) to make a mutation spectrum, run the script on a nucleotide list file (e.g., compare\_A\_B.csv). For example:

```
python count_substitution_types_A_to_B.py --compare compare_A_B.csv
```