Quickstart guide to analyzing antibody-pathogen interactions using iCn3D and Nextstrain.org

In order to analyze pathogen-antibody interactions, the following conditions must be met: the sequence information for the pathogen is available, the pathogen proteins of interest have a high level of mutability, and structures neutralizing antibodies bound to pathogen proteins (PDB files) are available.

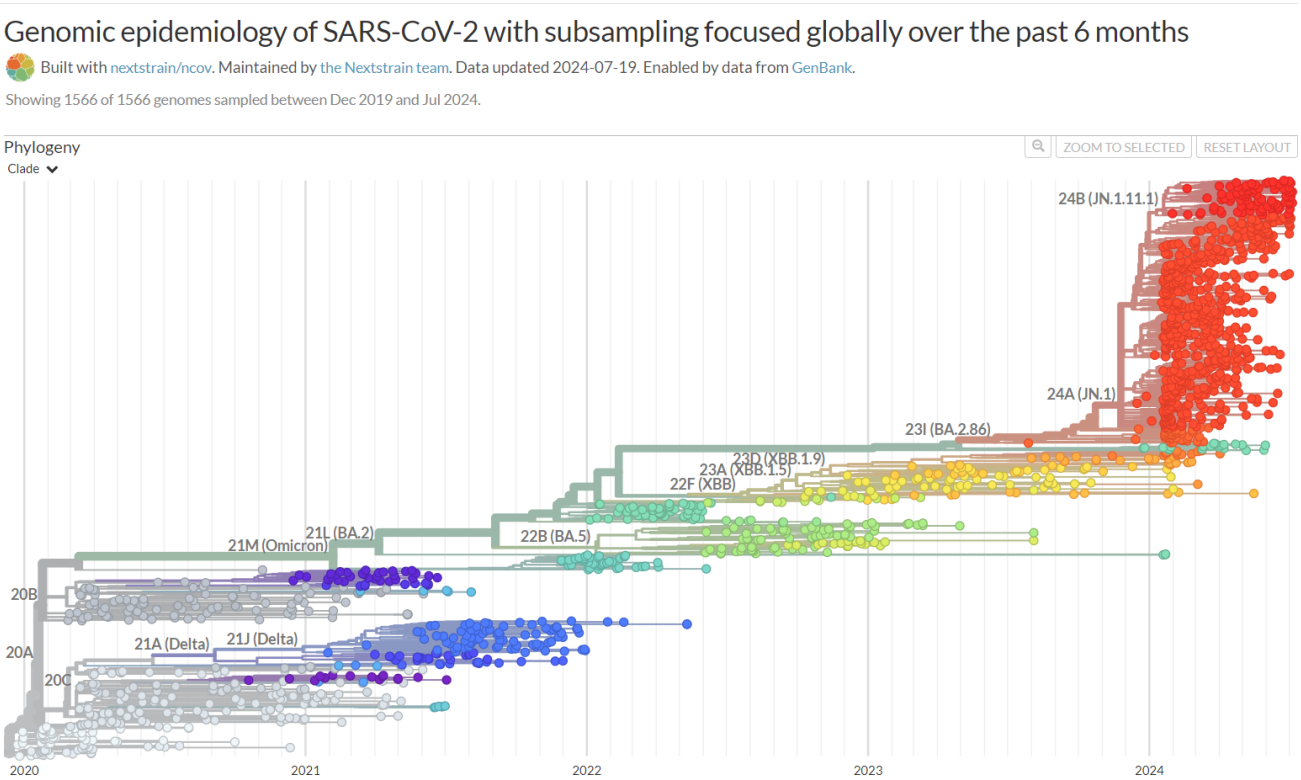
Full detailed instructions are available at https://qubeshub.org/publications/2913/1 from S. Porter, U. Hilgert, E. Lannan, and M. Stieber, "Evaluating the potential for immune escape: how likely is an antibody to protect against a specific SARS-CoV-2 variant?," QUBES Educational Resources (2022).

Nextstrain.org

Nextstrain.org is an open source research project that tracks real-time pathogen evolution. It can be a useful website for the first two requirements for selecting a pathogen of interest: pathogen sequence information and the level of pathogen mutability.

At the time of this writing (July 2024), 11 pathogens are currently being tracked on Nextstrain.org: SARS-CoV-2, Seasonal Influenza, Avian Influenza, Mpox, RSV, Dengue, Ebola, Measles, Zika, Mumps, and Enterovirus. https://nextstrain.org/pathogens

Nextstrain.org provides information on the spread and evolution of these selected pathogens. An example of the evolution and how the information is presented on the site can be found in Supplemental Figure 1. Selecting one of the circles provides a user with an array of information on that sample including the location, date, country, host, lineage, sequence changes, and in many cases, GenBank accession information.



Supplemental Figure 1: Genomic epidemiology of SARS-CoV-2 from December 2019 - July 2024. Each circle represents a sample submitted to a sequencing facility, and the samples are grouped according to sequence and lineage.

SAbDab

SAbDab is a structural antibody database (https://opig.stats.ox.ac.uk/webapps/sabdab-sabpred/sabdab) built by the Oxford Protein Informatics Group (OPIG) under an open-innovation agreement, and data is available under a CC-BY 4.0 license. SAbDab can be a useful website for identifying neutralizing antibody structures (PDB files) for the pathogen of interest.

On this site, you can search by a number of attributes, including species and antibody type. We recommend downloading all structures via the “search structures” then “get all structures” options, and then searching for your pathogen or protein of interest within the downloaded file for the most inclusive results.

iCn3D

iCn3D is a free, open-source, web-based structure application (https://www.ncbi.nlm.nih.gov/Structure/icn3d/). iCn3D has two major benefits for structure analysis in a classroom setting. First, it is a web-based program, so it does not require students or instructors to download any files in order to use the structure viewer. Secondly, it is the only application that allows the

users to share links to the annotated structure models, which is an important feature for evaluating student work and sharing files directly with students.

Users can input structure information on the iCN3D site in the format of PDB, MMDB, or AlphaFold UniProt IDs. Note that it is possible the structure information may contain multiple antibodies, but by default, the Biological Unit (minimum form) will be shown. If users prefer to load the entire structure, potentially including multiple antibodies, select Load Asymmetric Unit (All Chains) instead. After loading the structure, clicking on the PDB ID within the title of the structure will open a new tab and take users to the NCBI structure summary website. Information available on this site includes the original paper describing the structure, the experimental method of isolation, and number and interactions of chains in the original structure.

After loading the desired PDB file, many tools are available to users. For example, the program can identify amino acids within a certain distance range via the "Select by distance" command to view the amino acids that are likely used for binding and interacting, align two sequences using NCBI accession numbers (File→ align → sequence to structure), and view annotations (Analysis → Seq and Annotations). Faculty considering using iCn3D for this protocol and analysis are strongly encouraged to work through the detailed instructions, student activities, and answer key available on QUBES https://qubeshub.org/publications/2913/1.